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SATO, Masakazu and HIRANO, Tamotu :
Enzyme Chemical Investigation of Formosan Snake Venoms



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Professor **Bunichirô AOKI**, Dean of the Faculty (*ex officio*)

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On the Stability of Dipeptidase in the Extract of Muscle of Snake *Natrix annularis* (Hallowell)

Masakazu SATO

(With 6 Text-Figures)

(Accepted for publication 1933.)

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INTRODUCTION

In the year 1929, Linderström-Lang reported that, in the water extract of pigs' intestinal mucous membrane, glycylglycine (=GG) cleavage is more unstable than leucylglycine (LG) cleavage, presuming indirectly from the fact that the ratio Q of both enzyme actions ($=X_{AG}/X_{LG}$) of the water extract is smaller than that of the glycerine extract. (4). Later, it was similarly shown by Linderström-Lang and by the present author that, in its water extract, alanyl-glycine

(=AG) cleavage is more unstable than LG cleavage. (6). We observed further a similar relation for the dipeptidase of green malt. (6). Such relations were also revealed by the experimental results obtained by Euler, Myrbäck and Myrbäck with green malt. (1). But, later, in the year 1931, when the author directly investigated the stability of dipeptidase of green malt, quite a contrary result was obtained in its water extract, i. e., in this case, LG cleavage appeared far more unstable than AG cleavage. It was also found that, in its 88% glycerine extract, LG cleavage is very stable while, on the contrary, AG cleavage is extremely unstable. The latter phenomenon is not also identical with the previously ascertained fact that the dipeptidase is generally stabilized in its concentrated glycerine extract.

From these abnormal phenomena, the author presumed that AG and LG cleavages can vary their stability quite independently of each other, and that the direction of the independent change should be varied according to the enzyme material, the various treatment of the materials before extraction, or to the various kind of solvent etc., used, thus taking its course as possibly being sometimes activated or sometimes inhibited. For it is possible that, in the extracts of various natural enzyme materials, there may often be contained varying concomitant substances such as, stabilizers and unstabilizers or activators and inhibitors etc., and these concomitant substances would probably exercise various independent influences upon the stability of both enzymes.

Starting from the above presumption, the author undertook to investigate the stability of dipeptidase in the extracts of various natural enzyme materials such as the muscle and liver of snake or tortoise, etc. Of all the experimental results thus obtained, the author discovered in the extract of the muscle of snake several phenomena which were most interesting and supported his presumption beyond doubt. Therefore, the author wishes here to deal with the stability of dipeptidase mainly in relation to the case above mentioned, while, the results of the investigations of other cases, will be communicated in separate papers.

The author would like to tender his cordial thanks to Prof. Dr. K. Oshima, Dean of the Faculty of Science and Agriculture in the Taihoku Imperial University, for the interest he has taken in this work.

EXPERIMENTAL PART

A. Preparation of Enzyme Solutions

1. *Extracts prepared from fresh muscle of snake*

Fresh muscle of snake was ground into porridge-like condition in a meat mincer, and X g. of this ground muscle was mixed with sufficient glycerine and water to give a total volume of 2X cc and a glycerine concentration of 58%, 24% or 0%. The mixtures were well ground in a mortar, made to stand for some time, and were then centrifuged and filtered until the solutions separated were clear. The water content of the fresh muscle used averaged 73.81%.

2. *Extracts prepared from dried muscle of snake*

a. *Dried muscle of snake*

The method for the preparation of dried muscle of snake was essentially the same as that employed by Willstatter and Waldschmidt-Leitz for drying pancreas. (10).

1 kg. of the ground muscle was treated twice with 2 litres of acetone, then twice with a mixture of 1 litre of acetone and 1 litre of ether, and finally twice with 2 litres of pure ether. Each operation was performed rapidly and the suspensions were filtered immediately without standing. The product thus obtained was dried between filter papers and filtered through a sieve of 1 mm. mesh and the finely sifted powder was kept for use in a vacuum desiccator.

b. *Extract prepared from dried muscle of snake*

X g. of dried muscle of snake was mixed with sufficient glycerine and water to give a total volume of 5X cc. and a glycerine concent-

ration of 58%. The mixture was well ground in a mortar, was left to stand for some time, and then centrifuged and filtered.

B. Preparation of Substrate Solutions

1. Substrates

AG and LG were racemic. They were prepared according to Fischer's method (2) and analysed for carboxyl groups, amino groups and total nitrogen. The peptides were recrystallized at least once.

2. Substrate Solutions

Substrate solutions were prepared in the same way as described in previous papers (5) (6) (7) (8) (9), i. e., 0.2 mol. LG or AG+0.15 mol. ammonia+0.25 mol. ammonium chloride in 1 litre, P_H 8.0.

C. Determination of Stability

The extracts were kept standing in 1% toluene at 0°C or 40°C for varying lengths of time at the natural P_H or at the varying P_H which was regulated with ammonia or acetic acid, etc., and the enzyme activities were measured at varying intervals. The P_H of each extract was determined at 18°C by the colorimetric or quin-hydronic method.

D. Determination of Enzyme Activities

The method used here was the semi-micro titration method as devised and modified by Linderström-Lang and the author. (5) (8). The method was based upon the same principle as the alcohol titration method of Willstätter and Waldschmidt-Leitz (10). In the following, only important points are to be noted. The total volume of the digestion mixture is 5 cc. From this mixture, every 2 cc. are pipetted off before and after digestion and the increase of carboxyl groups per 2 cc. of the digest is measured by the above titration method. The conditions for digestion are:— substrate concentration

0.1 mol., glycerine concentration 15%, P_H 8.0 (Ammonia-ammonium chloride buffer), digestion at 40°C for 1 hour. For the determination of P_H -activity-curves, the P_H of the digestion mixture was regulated by the addition of ammonia or acetic acid, making the same total volume of the digest. The P_H of each digestion mixture was measured at 40°C by the colorimetric method.

E. Symbols

For the sake of reference, I have collected here the symbols used in the preceding and following sections.

AG=alanyl glycine, LG=leucyl glycine.

XAG, XLG=number of carboxyl groups formed by the splitting up of the peptide (AG or LG) under the given conditions, expressed in cc of $n/20$ KOH per every 2 cc of digestion mixture.

C_E =enzyme concentration reduced to grams of dried muscle used to prepare that amount of enzyme extract per every 2 cc of digestion mixture.

Table 1

Survey of the enzyme extracts prepared.

Number of extract	Date of preparation	material extracted	a g. material extracted in		P_H of original extract	
			conc. of solvent	volume of mixture	C	Q
1a	30/9 1932	fresh muscle	58% G	2a cc	6.20	6.25
1b	"	"	24% G	"	6.45	6.54
1c	"	"	W	"	6.55	6.63
2	25/10	"	58% G	"	—	—
3	1/11	"	58% G	"	—	—
4	24/11	"	W	2a	—	—
5	13/12	"	W	2a	—	—
6	10/11	dried muscle	58% G	5a	—	6.07

Note:— G=glycerine, W=water, C=colorimetric, Q=quinhydrone. Extracts 1a, 1b, 1c, and 4 were used for the stability experiment at 0°C. and 2, 4, for the stability experiment at 40°C. Extracts 3, 5 and 6 were used for obtaining P_H -activity-curves.

F. Enzyme Extracts Employed

Table 1 contains a survey of the extracts of muscle of snake employed in the present investigations and the particular data relating to their preparation.

G. Experimental Results

The various phenomena relating to the stability of dipeptidase in the extract of muscle of snake are clearly shown in the following tables and figures, therefore, only important points need to be explained here.

Fig. 1 shows the preliminary stability curves which illustrate the change in AG as well as LG cleavages in the extract when it was prepared by the extraction with 58%, 24% of glycerine or water and kept at 0°C. In this case, both cleavages have a similar tendency to be stabilized as the concentration of glycerine in the extract increases, but the degree to be stabilized is different according to the respective kind of cleavage.

Fig. 2 shows the stability curves which illustrate the change of both cleavages in a 58% glycerine extract when the extract was regulated to the varying P_H and kept standing at 40°C. In this case, a quite contrary independent change at each P_H , is clearly observed such as an increase in the activity of LG cleavage corresponding, as the time goes by, to a decrease in the AG cleavage. In view of the fact that the P_H of the extract is not practically changed before and after standing, it is evident that this independent change is not due to the change of the P_H of the extract but to the influence of other concomitant substances present in the extract.

Fig. 4 illustrates the comparison between the P_H -activity-curves of both cleavages when the 58% glycerine extract of fresh muscle of snake was kept standing at 40°C and at a regulated P_H 7.0 for 60 hours. In this case, the P_H -activity-curve of the AG cleavage becomes distinctly lower after standing, while, that of the LG cleavage becomes remarkably higher, especially at its optimal P_H zone.

Thus, a conclusive evidence was obtained that the stabilities of both cleavages are variable quite independently of each other. According, however, to the results of another experiment of ours (13) made with the fresh muscle of tortoise, this relation is quite different, both cleavages being quite stable with neither activation nor inhibition.

Fig. 5 illustrates the comparison between the P_H -activity-curves of both cleavages before and after standing, when the 58% glycerine extract of dried muscle, which was prepared by the treatment with acetone and ether, was kept standing at 40°C and at its natural P_H for 60 hours. In this case, the P_H -activity-curves of the LG cleavage coincide completely before and after standing, while that of the AG cleavage becomes distinctly lower after standing. According to this, it is evident that the relation between the stability of both cleavages is changed even by the simple process of drying the enzyme materials.

Fig. 3 shows the stability curves which illustrate the change of both cleavages in a water extract of fresh muscle of snake when kept standing at 40°C and at its natural P_H . According to this, at the initial stage of standing, the AG cleavage is more unstable than the LG cleavage, though the activities of both cleavages decrease rather rapidly. According to another experiment of ours (13), made with the water extract of fresh muscle of tortoise, this relation is reversed, i. e., the LG cleavage is more unstable than the AG cleavage, while, a different case also occurs in the water extract of dried powder of the liver of tortoise, where the activities of both cleavages are equally stable even from the beginning of the standing period. As shown from the same fig. 3, during the final stage of standing, the activities of both cleavages become, respectively, very constant and extremely stable. It was strictly and distinctly proved that these constant activities are not due to the autolysis of the enzyme extract. (Table 4). Such a phenomenon was also often observed in another of our experiments, for example (12), in the water extract of the fresh liver of snake, etc. This phenomenon should be considered as either the condition in which the stabilizers and unstabilizers, etc., attained to their equilibrium or that in which the unstable enzyme

disappeared and only the stable enzyme remained.

Fig. 6 illustrates the comparison between the P_H -activity-curves of both cleavages when stabilized by keeping the water extract of fresh muscle of snake standing at 40°C for 70 hours. According to this, it may be noticed here that there is a marked difference between the forms of the curves, though the optimal P_H for both cleavages is equally at P_H 8.0.

CONCLUSION

(1) In the extract of muscle of snake, especially in a 58% glycerine extract of the fresh muscle, the stability of both dipeptidase actions, the AG and LG cleavages are variable quite independently of each other.

(2) The direction of the independent change is varied according to the solvent, the previous treatment of the enzyme materials, or the various period of standing, etc. This should be considered as due to the fact that, according to varying conditions named, there are contained in the extract various kind of concomitant substance such as stabilizers and unstabilizers or activators and inhibitors, etc., in varying quantities and the influence of these concomitant substances upon the AG cleavage differs in various ways from that exercised upon the LG cleavage.

(3) According to other experiments of ours (12) (13), this relation between the stability of both cleavages is also remarkably different according to the various kind of enzyme material used. This should be considered as due to the same reason as that above stated.

(4) The problems relating to the "Einheitlichkeit" of the dipeptidase were fully discussed, from the standpoint of the affinity between the enzyme and the substrate, by Grassmann and Klenk with reference to yeast, etc., (3) and by Linderström-Lang with reference to intestinal mucous membrane (7) and by the author with reference to green malt. (8). According to the hypothesis of Grass-

mann and Klenk, there is only one dipeptidase in the nature but this dipeptidase has affinities for various dipeptides. However, this hypothesis could not be supported by the experiments of either Linderström-Lang or the author. According to the present investigation it was conclusively proved from the standpoint of the stability of dipeptidase that AG and LG cleavages must be due to two specific enzyme actions. At the present stage of enzyme chemistry, we must be satisfied with this conclusion, though it is another problem whether these two specific acitons are due to two kind of real enzyme body or not.

Fig. 3.

Stability-curves of dipeptidase in water extract of fresh muscle of snake at natural P_H and at 40°C . The curves correspond to the figures in Table 4.

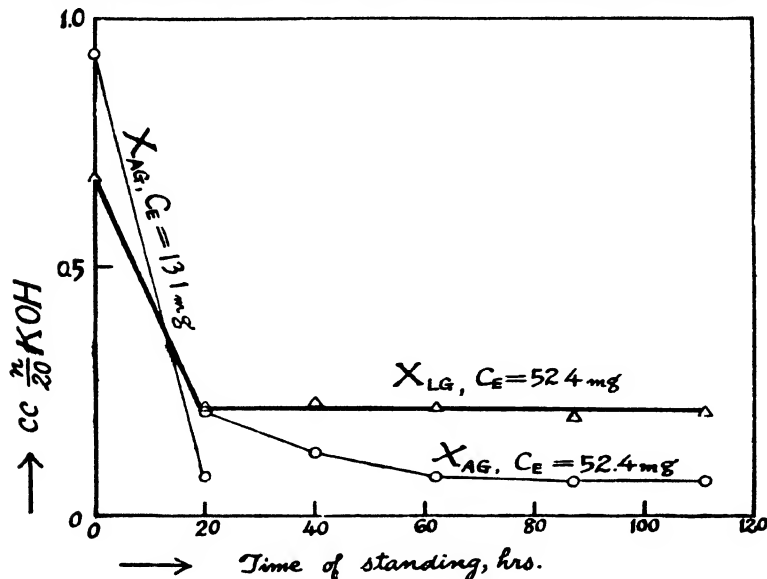


Fig. 4.

Comparison between the P_H -activity-curves of dipeptidase before and after keeping 58% glycerine extract of fresh muscle of snake standing for 60 hours at a regulated P_H 7.0 and at 40°C .

The curves correspond to the figures in Table 5.

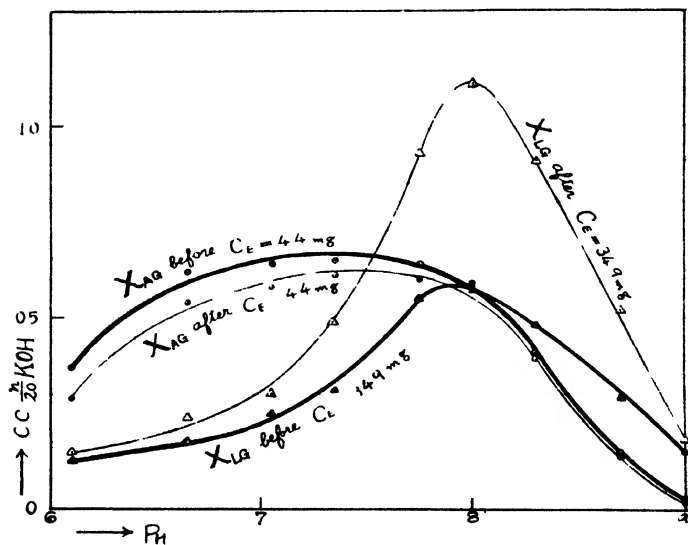


Fig. 5.

Comparison between the P_H -activity-curves of dipeptidase before and after keeping 58% glycerine extract of dried muscle of snake standing for 60 hours at 40°C. The curves correspond to the figures in Table 6.

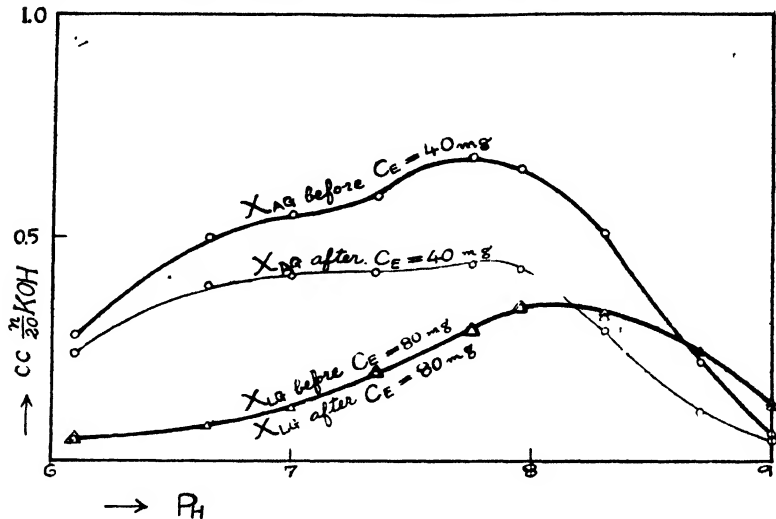


Fig. 6.

Comparison between the P_H -activity-curves of dipeptidase stabilized by keeping water extract of fresh muscle of snake standing for 70 hours at 40°C.

The curves correspond to the figures in Table 7.

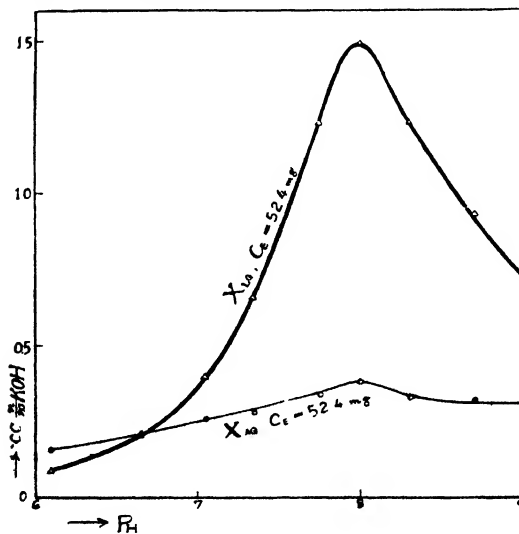


Table 2.

Stability of dipeptidase in the extract of fresh muscle of snake at natural P_H and at 0°C . Extract 1a, 1b, 1c.

Nature of extract	P_H of extract		Time of standing, days	CE mg for		X_{AG}		X_{LG}	
	C	Q		X_{AG}	X_{LG}				
58%G	6.2	6.25	4	6.5	52.4	1.11	1.12	0.98	0.98
24%G	6.45	6.54	"	"	"	1.12		0.97	
						0.88	0.88	1.06	1.06
W	6.55	6.63	"	26.2	"	0.87		1.06	
						1.38	1.38	0.71	0.71
						1.38		0.70	
58%G	6.2	6.25	11	6.5	"	1.08	1.08	1.12	1.12
						1.08		1.12	
24%G	6.45	6.54	"	"	"	0.57	0.57	0.96	0.96
						0.57		0.96	
W	6.55	6.63	"	26.2	"	0.45	0.46	0.36	0.37
						0.47		0.37	

Table 3.

Stability of dipeptidase in 58% glycerine extract of fresh muscle of snake at varying P_H and at 40°C .

Extract 2. Kept standing at 0°C for 2 days before experiment. CE=4.4 mg for X_{AG} and 34.9 mg for X_{LG} .

P_H of extract		Time of standing, hours	X_{AG}	X_{LG}
C	Q			
6.2	6.25	0	0.68	0.83
7.0	7.01		0.67	0.80
7.4	7.30		0.66	0.80
8.0	8.01		0.63	0.77
8.4	8.64		0.56	0.74
		20	0.58	0.99
			0.59	1.04
			0.57	1.00
			0.47	1.00
			0.37	0.87
		40	0.52	1.14
			0.61	1.20
			0.51	1.13
			0.39	0.95
			0.22	0.67
		60	0.47	1.22
			0.55	1.29
			0.44	1.18
			0.23	0.79
			0.14	0.59

Table 4.

Stability of dipeptidase in water extract of fresh muscle of snake at natural P_H and at 40°C .

Extract 4. Kept standing at 0°C for one day before experiment.

Time of standing, hrs.	CE mg for		X_{AG}	X_{LG}
	X_{AG}	X_{LG}		
0	13.1	52.4	0.93	0.68
20	"	"	0.08	0.22
"	52.4	"	0.21	—
40	"	"	0.13	0.23
62	"	"	0.08	0.22
87	"	"	0.07	0.20
111	"	"	0.07	0.21

Test on the autolysis with the same extract as above.

Time of standing		CE	X autolysis	
at 40°C hours	at 0°C days			
87	—	52.4	0.00	0.01
"	—	"	0.01	0.01
—	12	"	—	0.00
—	14	"	0.01	0.01
—	"	"	0.01	0.01

Table 5.

Comparison between the P_H -activity relation of dipeptidase before and after keeping 53% glycerine extract of fresh muscle of snake standing for 60 hours at regulated P_H 7.0 and at 40°C .

Extract 3. Kept standing at 0°C for one day before experiment. CE=4.4 mg for X_{AG} and 34.9 mg for X_{LG} .

Time of standing at 40°C , hours	P_H before and after digestion		X_{AG}	P_H before and after digestion		X_{LG}
0	6.10	6.10	0.37	6.10	6.10	0.13
"	6.65	6.65	0.62	6.65	6.65	0.18
"	7.05	7.05	0.64	7.05	7.05	0.25
"	7.35	7.35	0.65	7.35	7.35	0.31
"	7.75	7.75	0.64	7.75	7.75	0.55
"	8.0	8.0	0.59	8.0	8.0	0.57
"	8.3	8.3	0.41	8.3	8.3	0.48
"	8.7	8.7	0.15	8.7	8.7	0.29
"	9.0	9.0	0.03	9.0	9.0	0.15
60	6.10	6.10	0.29	6.10	6.10	0.15
"	6.65	6.65	0.54	6.65	6.65	0.24
"	7.05	7.05	0.58	7.05	7.05	0.30
"	7.35	7.35	0.61	7.35	7.35	0.49
"	7.75	7.75	0.60	7.75	7.75	0.93
"	8.0	8.0	0.58	8.0	8.0	1.11
"	8.3	8.3	0.39	8.3	8.3	0.91
"	8.7	8.7	0.14	8.7	8.7	0.52
"	9.0	9.0	0.02	9.0	9.0	0.18

Table 6.

Comparison between the P_H -activity-relation of dipeptidase before and after keeping 58% glycerine extract of dried muscle of snake standing for 60 hours at 40°C.

Extract 6. $C_E=40.0$ mg for X_{AG} and 80.0 mg for X_{LG} .

Time of standing, hours.	P_H before and after digestion		X_{AG}	P_H before and after digestion		X_{AG}
0	6.10	6.10	0.28	6.10	6.10	0.05
"	6.65	6.65	0.50	6.65	6.65	0.05
"	7.00	7.00	0.55	7.00	7.00	0.12
"	7.35	7.35	0.59	7.35	7.35	0.20
"	7.75	7.75	0.68	7.75	7.75	0.29
"	7.95	7.95	0.65	7.95	7.95	0.34
"	8.3	8.3	0.51	8.3	8.3	0.33
"	8.7	8.7	0.22	8.7	8.7	0.25
"	9.0	9.0	0.06	9.0	9.0	0.13
60	6.10	6.10	0.24	6.10	6.10	0.05
"	6.65	6.65	0.39	—	—	—
"	7.00	7.00	0.41	—	—	—
"	7.35	7.35	0.42	7.35	7.35	0.20
"	7.75	7.75	0.44	7.75	7.75	0.29
"	7.95	7.95	0.43	7.95	7.95	0.34
"	8.3	8.3	0.29	8.3	8.3	0.32
"	8.7	8.7	0.11	—	—	—
"	9.0	9.0	0.05	9.0	9.0	0.12

Table 7.

Comparison between the P_H -activity-relation of dipeptidase stabilized by keeping water extract of fresh muscle of snake standing for 70 hours at 40°C.

Extract 5. $C_E=52.4$ mg for X_{AG} and X_{LG} . 6 hours digestion at 40°C.

P_H before and after digestion		X_{AG}	P_H before and after digestion		X_{LG}
6.10	6.10	0.16	6.10	6.10	0.09
6.65	6.65	0.21	6.65	6.65	0.21
7.05	7.05	0.26	7.05	7.05	0.40
7.35	7.35	0.28	7.35	7.35	0.66
7.75	7.75	0.34	7.75	7.75	1.23
8.0	8.0	0.38	8.0	8.0	1.49
8.3	8.3	0.33	8.3	8.3	1.23
8.7	8.7	0.32	8.7	8.7	0.93
9.0	9.0	0.31	9.0	9.0	0.72

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INVESTIGATION ON THE PROTEOLYTIC ENZYMES IN GREEN MALT.[§]

(With 17 Text-Figures)

Masakazu SATO.

(Accepted for publication, April 24, 1934)

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§ This is a collection of some of my works which have been carried out during 1928-1931 at the Carlsberg Laboratory, Copenhagen, Denmark, under the direction of Professor S. P. L. SÖRENSEN and Dr. K. LINDERSTRÖM-LANG and were already published in several Journals (15, 16, 20, 21). This is published by the permission of Prof. SÖRENSEN and Dr. LINDERSTRÖM-LANG, to whom my hearty thanks are due.

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(A)

ON THE DETERMINATION AND SEPARATION OF THE PROTEOLYTIC ENZYMES IN GREEN MALT

THEORETICAL PART

In a previous paper,¹⁹⁾ MILL and LINDERSTRÖM-LANG have given a preliminary communication on the proteolytic enzymes of green malt. From this it appears that extracts of malt contain at least two well-defined proteases, one peptidase, and one proteinase (if we use the terminology of GRASSMANN²⁰⁾). The present paper contains researches on these enzymes, and proposes methods for the determination and separation of them.

Very few researches have been made on the proteases of malt. ABDERHALDEN and DAMMHAHN²¹⁾ were the first to demonstrate the presence of a peptidase in sprouting barley. MILL and LINDERSTRÖM-LANG who did not know of this paper gave a short review of the later literature and pointed out the importance of a comparison with the corresponding enzymes of yeast, which have quite recently been investigated by GRASSMANN, whose work must be said to be pioneer work in this domain. The resemblance between the proteases of yeast and those of malt is not, however, considerable. The PH-optimum for the proteinase of malt thus lies at 4.3, while for the proteinase of yeast (activated with hydrogen cyanide) Grassmann and Dyckerhoff give PH 7.0 (both, in the cleavage of edestin). According to the present researches, in solutions free from proteinase and phosphate, the peptidase of malt has the PH-optimum 8.6, while for the corresponding yeast enzyme DERNBY²²⁾ and WILLSTÄTTER and GRASSMANN²³⁾ give 7.8,—in a previous paper¹⁹⁾ MILL and LINDERSTRÖM-LANG found the optimum 7.6 in phosphate buffer for the peptidase of malt.

This is due to the fact that, in phosphate buffer, the optimum displaces considerably in the acid direction, as far as the malt peptidase is concerned.

All the researches have been made on extracts of malt prepared according to MILL and LINDERSTRÖM-LANG, but dialysed before use in order to remove phosphates, which inhibit the hydrolysis of leucylglycine in an unusual degree. In these dialysed extracts of malt, the peptidase is very unstable, and even at 1° under toluene breaks up very quickly. The proteinase, on the other hand, stands storing fairly well. The simplest method of obtaining a proteinase solution free from peptidase is therefore to leave the water extract of malt to stand. The preparation of peptidase free from proteinase is considerably more difficult. Inquiry into the best method for separation of the two enzymes necessitated a stabilisation of the peptidase, and for this was employed glycerine, which, in a concentration of 44%, has a highly preserving influence. Most of the experiments have therefore been made with extracts containing glycerine, a fact which is not without importance for the determination of the proteinase as well as the peptidase, and necessitates correction for the inhibition of the activity of the enzymes by the glycerine. In the quantitative investigation of the displacement of the ratio between the peptidase and the proteinase under varying experimental conditions, the substrate edestin was used for the determination of the proteinase, and for the determination of the peptidase, as previously mentioned, leucylglycine; in a few cases also leucylglycylglycine. In the following is given a description of the methods which are based on FOREMAN, WILLSTÄTTER and WALDSCHMIDT-LEITZ's titrimetric determination of the increase of carboxyl groups in the substrate-enzyme-mixtures, under fixed conditions and with a suitably chosen time of experiment. The more detailed elaboration of the method falls fairly well in line with that employed by GRASSMAN.

For the separation of the enzymes were applied the methods developed by WILLSTÄTTER and his co-workers. It was found that, under the conditions investigated, aluminium hydroxide C_7^{30} is a less suitable

separation agent. In contrast to the results arrived at by WILL-STÄTTER and GRASSMANN GRASSMANN and HAAG¹⁰²⁸⁾ for the enzymes of yeast, according to which the peptidase (the dipeptidase) on being treated with aluminium hydroxide, and especially in acid solution, remains in the solution, while the proteinase (and the polypeptidase) are adsorbed, I have found that the most favourable (the most unequal) distribution as far as malt enzymes are concerned is obtained at neutral, faintly alkaline reaction; the proteinase here predominates in the precipitate; the peptidase in the solution. The separation is, however, far from being quantitative, it is only in extremely few cases that I have obtained residual solutions free from proteinase, and these were then always very poor in peptidase too. As a separation agents caolin holds about the same position as aluminium hydroxide. In acid solution the peptidase is adsorbed in a somewhat higher degree than the proteinase; at slightly alkaline reaction the adsorption of proteinase is considerably higher than that of the peptidase, but this latter condition cannot be directly utilized, because, curiously enough, an increase of the amount of the adsorption agent does not result in an increase of adsorption, either of one enzyme or of the other. Certain amounts, rather different it is true, remain in the residual solution. This difficulty might be surmounted by a combination of aluminium hydroxide and caolin adsorption, as a consecutive treatment with suitable quantities of the two adsorption agents yielded a residual solution which was, so to say, without proteinase, and which contained abt. 30% of the peptidase. I have, however, obtained still better results by employing ferric hydroxide, the behaviour of which is in its broad feature reminiscent of that of the other two adsorption agent, but which at PH 8 in a very short reaction interval is extremely selective in its adsorption of the proteinase (especially in presence of glycerine), the residual solution free from proteinase containing between 40 to 50% of the peptidase. This separation method should therefore be employed for continued experiments in the preparation of peptidase solution free from proteinase. By treatment of the ferric hydroxide adsorbates with phosphate (both

primary and secondary) as well as ammonia, the peptidase may comparatively easily be eluded, the proteinase somewhat less easily with phosphate, and with ammonia probably not at all.

EXPERIMENTAL PART

I. METHODS OF PREPARATION AND ANALYSIS

A. Methods of Preparation.

1. *Preparation of the Malt Extracts.*

The malt extracts were prepared according to MILL and LINDERSTRÖM-LANG³⁹⁾ from green malt stored in a refrigerating chamber at -10° , by a two hours' extraction from the well-ground malt with $\frac{4}{5}$ of its weight of water at 30° , and succeeding filtration. The filtrates obtained (stored at 1°) were, in contrast to what was previously done, subjected to a dialysis in collodion sacks under reduced pressure (cf.³⁹⁾) in order to remove phosphates, and the enzyme activity in the dialysed extracts, the volume of which varied between $\frac{1}{3}$ – $\frac{1}{4}$ of the volume before dialysis, was in most cases made stable by addition of the same volume of 88% glycerine. For further details, the reader is referred to p. 33.

2. *Preparation of the Adsorption Agents Employed for the Separation of the Enzymes.*

Aluminium hydroxide C_r was prepared according to WILLSTÄTTER, KRAUT and ERBACHER⁴⁰⁾; ferric hydroxide according to WILLSTÄTTER, KRAUT and FREMERY⁴¹⁾. Caolin was prepared from MERCK's "Bolus" for medicinal purposes according to Prof. STUMPF, WÜRZBURG, by suspending in dilute hydrochloric acid and followed by careful washing with water.

3. *Preparation of the Substrates Employed in the Enzyme Investigation.*

The edestin employed for the determination of proteinase was the same preparation as was used by MILL and LINDERSTRÖM LANG¹⁰⁾. Leucylylcine was prepared according to FISCHER⁵⁾ and analysed for carboxyl-, amino-groups and total-N.

B. Analytical methods.

1. *Determination of Proteinase.*

a) Method of Determination.

The following stock solution was prepared.

25 g of edestin + 0.30 mol of acetic acid + 0.09 mol of sodium acetate in 1000 cc.

The PH of this was 4.1, very nearly the optimum of the proteinase (cf.¹⁰⁾). 2.5 cc of this solution with sufficient water to make the volume after the addition of enzyme solution 5 cc, were placed in a small test tube provided with a rubber plug and warmed in the thermostat, which had a temperature of 40°. The required quantity of the enzyme solution under investigation, warmed to room temperature was then added, the time of addition being carefully taken on a stop watch. The reaction mixture was well shaken for about a minute, and in the course of the following 30 seconds 2 cc were carefully measured off into a good pipette. 1 minute 30 seconds after the addition of enzyme, the 2 cc were poured into a small 50 cc titration flask containing 10 cc of 96% alcohol, the mixture was shaken well, and the test tube was replaced in the thermostat at 40°. After the lapse of the time required for digestion, which was measured from the moment of the addition of enzyme, 2 cc were again removed with the same pipette (rinsed in ca. 0.5 cc of experimental liquid) and mixed in the same way with 10 cc of alcohol. Test experiments showed that this volume of alcohol sufficed to stop the enzyme action absolutely.

The alcohol mixtures with an addition of 0.4 cc of 0.5% thymolphthalein solution were then titrated with $n/20$ alcoholic potassium hydroxide solution, the titration being first carried to a rather strong bluish-green colour, then 20 cc of 96% alcohol was added, whereupon the titration was continued till the first bluish shade appeared. A microburette calibrated into $\frac{1}{50}$ cc and provided with a capillary waste tube and a lens was used. When the determination was carefully carried out, the carboxyl groups formed per 2 cc of experimental liquid during the time of experiment (FOREMAN,⁹ WILLSTÄTTER and WALDSCHMIDT-LEITZ¹⁰) could generally be determined with an accuracy corresponding to 0.01–0.02 cc $n/20$ KOH (cf. p. 32).

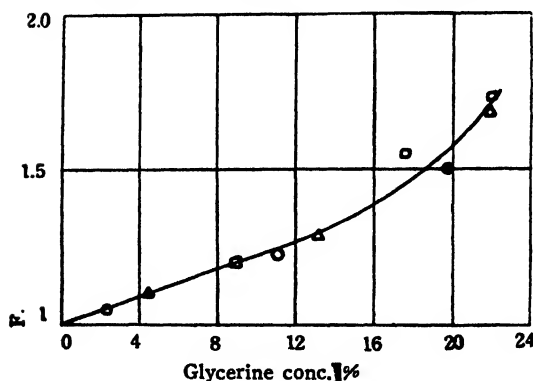
b) Definition of proteinase unit.

In the quantitative investigation of the purification and separation

Fig. 1.

Relation between glycerine error and glycerine concentration at the cleavage of edestin.

○: $c_E=0.1$. △: $c_E=0.2$. □: $c_E=0.4$.



of enzymes it is necessary to establish the relation between the digestion and the enzyme concentration. I have here limited myself to a purely empirical definition, based on an investigation of one of the malt extracts, viz. VIIG (cf. p. 35). As most of the enzyme solutions contained glycerine, I made a determination

of the influence of this substance upon the edestin cleavage, the result of which will be seen from table 1 and Fig. 1.

C_E : The number of cc of enzyme solution per 2 cc of digestion liquid.

x : The increase of carboxyl groups, expressed in cc of $n/20$ KOH per 2 cc of digestion liquid, in the course of 4 hours at 40° .

TABLE 1.

The hydrolysis of edestin with various quantities of malt extract VIIG,
and at different glycerine concentrations.

c_E	\times	F	Glycerine conc. in the digestion liquid %
0.1	0.27 (extr.)	1.00	0
"	0.26	1.04	2.2
"	0.22	1.23	11.0
"	0.18	1.50	19.8
0.2	0.44 (extr.)	1.00	0
"	0.40	1.10	4.4
"	0.34	1.29	13.2
"	0.26	1.69	22.0
0.4	0.76 (extr.)	1.00	0
"	0.63	1.20	8.8
"	0.49	1.55	17.6
"	0.44	1.73	22.0

F: The factor by which the \times found is to be multiplied in order to give the increase corresponding to the glycerine concentration 0.

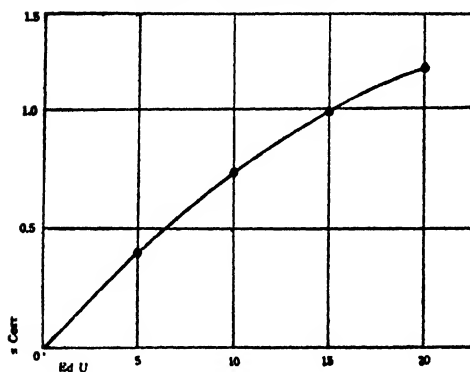
As VIIG in itself contained 44% of glycerine (cf. later on), I have had to extrapolate to zero glycerine concentration. This may be done graphically with sufficient accuracy. The manner in which the F-values (computed by simple division of \times (extr.) by the various \times values) group themselves round the correction curve, Fig. 1, justifies the method.

It will be seen that glycerine has an influence none the less on the velocity of the edestin cleavage. It is true that, in the present experiments, they have seldom been worked out with a higher concentration than 6% in the digestion liquids, the correction thus not being considerable. Yet it is sufficiently great not to be set aside and all the \times -values were therefore corrected by the help of Fig. 1 and they were multiplied by the F-value corresponding to the glycerine concentration.

The enzyme unit was defined on the basis of another experiment with VIIG, and was chosen arbitrarily, so as to make the content of units in 2 cc of digestion liquid equal to abt. 10 (with such quantities of dialysed malt extract as were generally employed). In the determination of the amount of enzyme the time of digestion was fixed at 4 hours (temp. 40') in the whole of this work. Table 2 and figure 2 show the connection between \times (see above) and the number of enzyme units per 2 cc of digestion liquid.

Fig. 2.

Relation between edestin cleavage and enzyme concentration.



Ed-U. Proteinase units (edestin units).

In Fig. 2, the Ed-U's are plotted against the values, corrected to zero glycerine concentration by help of the curve in Fig. 1.

According to the above definition malt extract VIIG therefore contains $5/0.2 \cdot 3/2 = 37.5$ Ed-U. According to this, the method for the determination of the Ed-U

of an enzyme solution is to make a digestion experiment as previously described, and with a suitable c_E , extending the time of experiment

TABLE 2.

Hydrolysis of edestin with different quantities of malt extract VIIG diluted with $1/2$ volume of water.*

c_E	\times	\times corr. to glyc. conc. o.	Ed-U pr. 2 cc digestion liquid.	glycerine conc. %
0.2	0.37	0.39	5	2.9
0.4	0.65	0.73	10	5.9
0.6	0.82	0.99	15	8.8
0.8	0.93	1.17	20	11.7

* This experiment was made ca. 1 month before the one described in table 1, so that the increases found there are somewhat slighter, the proteinase having become partly inactivated on standing (see later).

to 4 hours. From the \times found corrected for glycerine error (fig. 1) the number of Ed-U present in C_F , cc of enzyme solution can be graphically read in Fig. 2.

TABLE 3.
Contents of Ed-U in malt extract I.
Glycerine concentration o.

c_E	\times	Ed-U read in fig. 2.	Ed-U pr. cc malt extract 1.
0.05	0.29	3.7	74
0.1	0.56	7.4	74
0.15	0.77	10.7	72
0.2	0.98	14.8	74

Table 3 contains an example of the application of the method, viz. an experiment with malt extract I made without glycerine (see p. 33). The correspondence between the enzyme concentrations (last column) found for the various C_F -values is satisfactory.

I wish by the way, to emphasize that in all parallel investigations, I have, as far as possible, taken care that the glycerine concentration in the digestion liquids was the same. I also wish to point out that the endeavour to keep this factor constant and the necessity of being able to measure off the enzyme solution accurately involve dilution of the enzyme solutions present, which may make the C_F -values, stated later on and always referring to the original enzyme samples before the dilution, appear slightly arbitrary (see p. 33-35).

I have investigated the influence of still another factor, namely the phosphate concentration in the liquid under investigation. Since phosphates, as previously shown, and as will be further mentioned in the next section, have a highly inhibitive action on the digestive power of the peptidase, and since they are at the same time important elution agents in the enzyme experiments, for which reason the digestion liquids often contain phosphate, the question is vital, even though I have, as far as possible avoided the use of phosphate in this work. As shown in table 4, this source of error may, however,

be entirely ignored, as the stated concentrations of phosphate have not been exceeded in the present experiments.

TABLE 4.

Cleavage of edestin with malt extract II (see p. 33) with addition of different quantities of prim. potassium phosphate (PH 4.5).

c _E	Molarity of prim. potassium phosphate in the digestion liquid.	x
0.1	0	0.59
"	1/150	0.62
"	1/75	0.57
"	1/50	0.62

2. Determination of Peptidase.

a) Method of Determination.

The following stock solution was prepared:

0.2 mol of leucylglycine + 0.15 mol of ammonia + 0.25 mol of ammonium chloride in 1000 cc.

This solution had P_{H} 8.0, which according to MILL and LINDERSTRÖM-LANG¹⁰⁾ should correspond very closely to the optimum. The last section of this work will show that the optimum for peptidase solutions without phosphate and proteinase lies somewhat more in the alkaline direction, namely at 8.5-8.6 so that with this stock solution experimental solutions have not the optimal P_{H} . As the optimum, however, is not marked by any broader maximal zone, P_{H} 8.0 may just as well be chosen as a basis for the peptidase determination.

For the rest the mode of procedure was the same as described under the proteinase determination; 2.5 cc of stock solution + water + enzyme solution, 5 cc in all, being mixed and serving to remove 2 cc of samples before and after digestion at 40°.

Checking and titration were likewise as mentioned before.

b) Definition of peptidase unit.

Here, as at the definition of the proteinase unit the empirical base was malt extract VIIG. The time for cleavage was 1 hour in all cases where nothing else is expressly mentioned. The influence of the glycerine concentration was investigated, and the factor F was calculated as before. Tables 5 and 6, Figs. 3 and 4 show the glycerine error, and the connection between the increases and the amounts of enzyme, the malt extract VIIG,

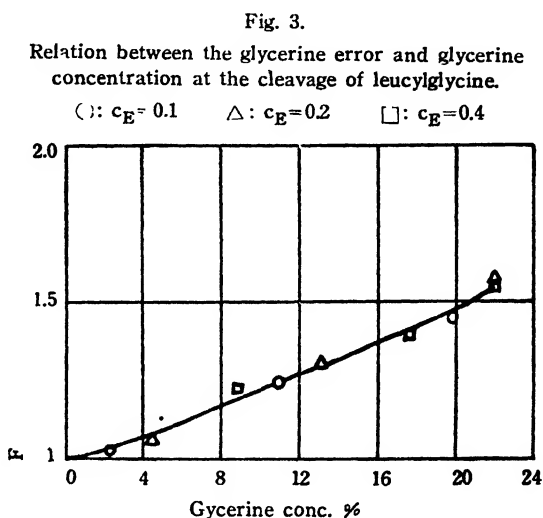


TABLE 5.

Cleavage of leucylglycine by various quantities of malt extract VIIG and at varying glycerine concentrations.

c_E	\times	F	Glycerine concentr. in the digestion liquid.
0.1	0.42 (extr.)	1.00	0
"	0.41	1.02	2.2
"	0.34	1.24	11.0
"	0.29	1.45	19.8
0.2	0.88 (extr.)	1.00	0
"	0.83	1.06	4.4
"	0.67	1.31	13.2
"	0.56	1.57	22.0
0.4	1.60 (extr.)	1.00	0
"	1.30	1.23	8.8
"	1.14	1.40	17.6
"	1.04	1.54	22.0

in accordance with the definition of the proteinase unit, being arbitrarily supposed to contain 37.5 peptidase units per cc.

C_E : The number of cc enzyme solution per 2 cc of digestion liquid.

\times : The increase of carboxyl groups, expressed in cc of $n/20$ KOH per 2 cc of digestion liquid; digestion during 1 hour at 40° .

TABLE 6.

Cleavage of leucylglycine by different volumes of malt extract VIIG, diluted with $1/2$ volume of water.*

C_E	\times	\times corr. to glycerine conc. 0.	LG-U pr.2 cc digestion liquid.	Glycerine conc. %
0.2	0.58	0.61	5	2.9
0.4	1.13	1.27	10	5.9
0.6	1.45	1.72	15	8.8
0.8	1.63	2.06	20	11.7

* This experiment was made ca. 1 month before the one described in Table 5, so that the increases found there are somewhat smaller, corresponding to a partial inactivation of the peptidase through standing.

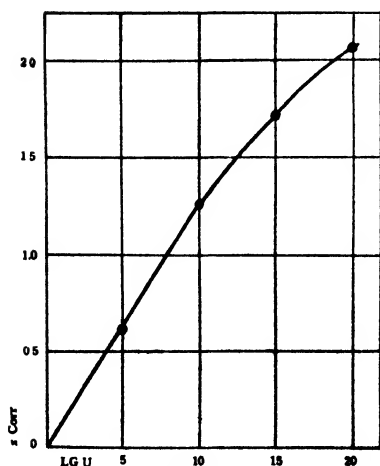


Fig. 4.

Relation between leucylglycine
Cleavage and enzyme
concentration.

LG-U: Peptidase units (leucylglycine units).

F: The factor by which the \times found must be multiplied in order to give the increase corresponding to the glycerine connection 0.

In Fig. 4 the number of LG-U (abscissae) are plotted against the \times values (ordinates) corrected to glycerine concentration 0 by means of the curve in Fig. 3.

The way of determining the number of LG-U in an enzyme solution is exactly analogous with that of determining the proteinase

units, only here the time of experiment is one hour. The influence of the glycerine concentration is about the same too and a correction is therefore necessary. In parallel experiments the glycerine concentration was kept constant if possible.

A similar method was chosen when correcting for the influence of a much more important factor: the phosphate concentration in the digestion liquid. As shown by MILL and LINDERSTRÖM-LANG this is of very great importance, and I have therefore endeavoured, firstly, as far as possible to avoid the use of phosphate solutions in my experiments (the malt extracts being freed from phosphate by the dialysis), and secondly, to eliminate in a suitable way, the influence of the phosphates during the determination of peptidase in the eludates obtained in the few experiments where phosphates were used for the elution of the peptidase from its adsorption compound with $\text{Fe}(\text{OH})_3$. Hence the following procedure was taken. Before the adsorption experiment I fixed the rate of hydrolysis (\times) of leucylglycine by the malt extract employed for the experiment with addition of the quantity of phosphate (of P_{11} 8) which the digestion liquid would contain at the peptidase determination in the eluate. A simple division by this \times value of the value found without addition of phosphate and with the same C_{11} , will then give the factor by which the \times value of the eluate must be multiplied in order to obtain a corrected carboxyl increase, which eventually by help of Fig. 4 may be converted into peptidase units. This method was chosen because I did not succeed in finding any general quantitative connection between the phosphate concentration and the rate of reaction, the various malt extracts showing individual differences, which would be of no importance with small corrections, but which cannot be ignored in this case, as the correction amounts to 200–300%. I have not subjected the question to any particular investigation, since as stated above, I have only in very few cases had solutions containing phosphate for determination.

3. Control Determinations of the Zero Value of the Cleavage.

Remarks as to the accuracy of the experiments.

On p. 24 it is mentioned that the determination of \times may be made with an accuracy of 0.02 cc n/20 KOH. According to this, then only enzyme solutions that yield increases below 0.02 are to be regarded as enzyme-free. This is correct in the case of the peptidase determination, control experiments with substrate buffer without enzyme as well as with enzyme+buffer of P_H 8 without substrate always yielding increases below this value. But in the proteinase determination factors appear as to which I am not quite clear, but which cause even enzyme solutions free from proteinase (see below) to give increase of 0.04 cc of n/20 KOH with acetic acid-sodium acetate solution of P_H 4.1 without edestin. I have not considered it necessary to enter this correction into all the values of the proteinase determinations, as its quantitative value may be disputed, but I have thought it justifiable to regard all enzyme solutions, yielding increase of 0.05 cc n/20 KOH and less, as devoid of proteinase. It must be emphasised that in the separation of enzymes in such undefined solutions as malt extracts, a certain doubt about the removal of the last trace of an enzyme must always make itself felt, and that, at the present stage of experimental technics the demand for completeness of separation should not be too great.

I likewise wish to point out that sometimes, without being able to find the cause, deviations between double determinations, considerably larger than 0.02 cc n/20 KOH, 0.04 or the like may appear. The peptidase determination especially may exhibit quite a number of such deviations. Generally, however, considering the great P_H -dependence of the enzyme action good correspondence in the results is obtained in this case, too.

4. The determination of P_H .

The P_H -measurements were partly colorimetric, partly quinhydrone. Whereas the determination of the P_H -values of the malt

extracts and the adsorption mixtures was made at 18-20°, the hydrogen-ion measurement of the digestion liquids was carried out at 40° according to the colorimetric method, and in the following manner: The test tubes containing the standard solutions (the P_{H^+} values of which were corrected to 40°) as well as the experimental liquids were immersed in the thermostat, which was provided with a glass-pane in front of them. Behind the test tubes was placed a plate of frosted glass, and behind this again a daylight lamp. Owing to the colour of the malt extracts a measurement according to Walpole's principle was often necessary. The method made it possible to determine P_{H^+} with an accuracy of 0.05-0.1 (for the rest see p. 75). No correction for the glycerine content of the experimental liquids is required. The quinhydrone method was only applied to the malt extracts without glycerine; and the glycerine error on the three indicators employed in the colorimetric method; brom-thymolblue, phenolsulphonephthalein, and thymolblue, is below 0.1 in P_{H^+} in 22% glycerine solution.

II. PREPARATIONS EMPLOYED

A. The Malt Extracts.

Table 7 contains the data of all the malt extracts prepared. The extraction itself is alike for all the malt extracts (see p. 22), but differences appear in the type of malt used as well as in the duration of dialysis and the further treatment. Malt extracts I-IV were prepared from green malt of Danish barley from the Danish Spirit Factories in Slagelse and were placed in a refrigerating chamber (-10°) in September 1928, the malt extracts V-VII, on the other hand, were from Danish brewery malt from the Carlsberg Breweries, and were put into refrigerating chamber in April 1929. Experiments have shown no difference between the two kinds of malt, but, on the other hand, the experiments were not planned with more thorough-going investigations on this problem in view.

Malt extracts I and II, used for preliminary investigations, were not made stable with glycerine. From III, which was used in the

first glycerine-stabilisation experiments and for the adsorption experiments with aluminium hydroxide, and hence onward, the same volume of 88% glycerine was added to the dialysed extract with the exception of certain samples used for continued experiments on the stability of the enzyme. These glycerine solutions, which were kept at 1°, are called IIIG, IVG, VG etc. in the following, whereas the terms I, II, III, IV, etc. refer to the dialysed malt extracts. IV and V were used in the caolin- and ferric hydroxide adsorption experiments, and finally, VII, for the preparation of peptidase solution without proteinase, and for the series of investigations made on the P_H -optimum of the peptidase.

The enzyme concentrations stated in table 7, all refer to the undiluted dialysed malt extracts, although the determinations carried out in the case of III-VII have been made with the stabilised glycerine solutions. The \times values directly found, and given in the table, were corrected for the glycerine error, converted into enzyme units by help of the before-mentioned curves, and converted into LG-U or Ed-U per cc of original, dialysed malt extract. As regards the sample-taking I would point out that in all enzyme determinations—also in the following—in solutions derived from a malt extract, such C_E -values were chosen as corresponded to 0.4 cc of the raw malt extract before dialysis. By this I think I have in the best possible manner eliminated specific influence of the accompanying substances in the malt extract. (cf. the C_E -values employed in the determinations in table 7). I would also emphasise that with this method the glycerine concentration in determinations carried out with the same malt extract is always the same, and that the accuracy in measuring off the enzyme is facilitated, because the enzyme solution in question, diluted to a volume corresponding to that of the raw malt extract or to a whole multipulum of that of the raw malt extract, can be accurately pipetted off—0.4 cc per 2 cc of digestion liquid or 1 cc per 5 cc, the quantity produced at the beginning of the digestion experiment (see p. 23).

For the rest the following remarks apply to table 7: During

TABLE 7.
Survey of the malt extracts prepared.

Malt extracts ·

	I	II	III	IV	V	VIa	VIb	VII
Date of preparation. (Year 1929)	$\frac{2}{2}$	$\frac{1^4}{2}$	$\frac{11}{1}$	$\frac{8}{1}$	$\frac{21}{1}$	$\frac{1}{5}$	$\frac{1}{5}$	$\frac{17}{5}$
Standing before dialysis, days.	2	1	4	2	3	2	11	1
Duration of dialysis, days.	3	$4\frac{1}{2}$	$3\frac{1}{2}$	5	5	8	5	4
$V_h/V_a - \begin{cases} \text{before dialysis} \\ \text{after dialysis} \end{cases}$	4:1	4:1	3:1	4:1	4:1	4:1	4:1	3:1
P_H before dialysis	—	—	—	—	6.1	—	5.8	6.0
P_H after dialysis	—	—	6.4	—	4.5	—	4.2	6.0
X_{LG} after dialysis $c_E = 0.4, V_a/V_h$	1.16	1.17	0.92	1.06	1.07	0.00	0.08	1.13
X_{ED} after dialysis $c_E = 0.4, V_a/V_h$	0.56	0.63	0.57	0.69	0.73	0.49	0.55	0.64
LG·U per cc after dialysis	92	94	61	92	93	0	7	77
Ed U per cc after dialysis	73	84	64	106	113	70	81	74

the dialysis a dark coloured precipitate was deposited in all cases; it was removed and found to contain a small amount of the proteinase but no trace of the peptidase. No investigations have been made as to whether there is any connection between the amount of this precipitate and the P_H -variation observed during the dialysis of malt extracts V and VIb. The tendency of this variation is such that it may be explained by the presence in the malt extracts of acid protein substances, which alone condition the reaction of the solution and are partly precipitated when the phosphates dialyse out. It must, however, be noted that owing to their content of sugar, the

malt extracts are good nourishing substrates for bacteria producing acids, so that the displacement may probably be explained by a slight infection. Although it was formerly found that an addition of toluene seemed to make the enzymes stable, the experiments I have made in this work (see p. 38) do not seem to confirm this in all cases; for practical reasons I have therefore avoided the toluene addition during the dialysis except in the case of malt extract VII, when an accident with malt extract VIa and VIb had seemed to show that this precaution may sometimes be necessary even at $+1^{\circ}$, with dialysis of long duration or long standing before dialysis. Characteristic in this respect is the difference between P_{II} after the dialysis in malt extracts VIb and VII, when it is compared with the difference in content of peptidase. There is, however, hardly any direct connection between P_{II} and the stability of this enzyme (cf. malt extract V and the stability experiments given on p. 37-38). The problem requires a more minute investigation, as does also the variation in the content of peptidase as well as proteinase during the dialysis under different experimental conditions. In a later work I shall treat of various conditions, especially relating to the decomposition of peptidase with a special reference to the commenced differentiation of this enzyme or enzyme system mentioned in the introduction. For the rest the enzyme concentration in the dialysed extracts was satisfactory.

B. The adsorption agents.

2 preparations of aluminium hydroxide C_7 were employed; one Al_1 prepared $1/9$ 1928, contained 0.180 g Al_2O_3 per 10 cc; the other, Al_2 , prepared $18/3$ 1929, yielded 0.188 g Al_2O_3 ; ferric hydroxide, 0.349 g Fe_2O_3 per 10 cc, was prepared $18/4$ 1929, and finally caolin, which on incineration yielded a residue of 0.985 g per 10 cc, was prepared $9/4$ 1927.

TABLE 8.

Stability of peptidase and proteinase in water solution. Dialysed malt extract IV diluted with 3 volumes of water. Stored, first at $+1^{\circ}$ without toluene then at 30° with addition of toluene. In the determinations, $c_E-0.4$; glycerine conc. 0.

Days of standing	Temp. $^{\circ}$	LG-cleavage \times	Ed-cleavage \times
0	1	1.11	0.72
8	"	0.26	0.72
Further $\frac{3}{4}$	30	0.05	0.52

TABLE 9.

Stability of peptidase and proteinase in malt extract VG (p 33 containing 44 % glycerine, stored at 1° with toluene. In the determinations, $c_E-0.2$; glycerine conc. 4.4 %.

Days of standing	LG-cleavage		Ed-cleavage	
	\times	\times corr. for glyc. error	\times	\times corr. for glyc. error
0	1.07	1.16	0.73	0.80
3	1.05	1.14	—	—
12	1.07	1.16	0.62	0.68
13	—	—	0.63	0.69

TABLE 10.

Stability of peptidase in dialysed malt extract III, 1 diluted with the same volume of water, 2 diluted with the same volume of glycerine (IIIG).

Left to stand at 1° . In the determinations,
 c_E 0.267; Glycerine conc. 1 0, 2 59 %

Days of standing	P_H ca.	Water solution	Glycerine solution	
		\times	\times	\times corr. for glyc error
1	6.4	1.00	0.92	1.03
3	—	0.83	0.92	1.03
10	—	0.21	0.94	1.05
13	—	0.11	—	—
17	—	0.05	0.81	0.91

TABLE 11.

Stability of peptidase and proteinase in malt extract VII at 1°.

A. Diluted with 2 volumes of water, left to stand without toluene.

B. Diluted with 2 volumes of water, left to stand with toluene.

C. Diluted with 1 volume of 88% glycerine (VIIG) left to stand with toluene.

In the determinations for A and B, $c_E=0.4$, glycerine conc. 0; for C, $c_E=0.267$ (corresponding to the same amount of original malt extract), glycerine conc. 5.9%.

Days	A			B			C		
	P_H	\times (LG)	\times (Ed)	P_H	\times (LG)	\times (Ed)	P_H	\times corr. (LG)	\times corr. (Ed)
0	6.0	1.27	0.72	6.0	1.27	0.72	6.0	1.27	0.72
3	—	0.81	—	—	0.64	—	—	1.27	—
12	—	—	—	—	—	—	—	1.22	0.73
26	3.9	0.11	0.55	4.9	0.05	0.43	5.9	—	0.59
29	—	—	—	—	—	—	—	1.19	—

Note: After standing 26 days, solution A, smelt strongly acid and fermented, and the microscope showed plainly that it contained rod-shaped bacteria. Solution B on the other hand smelt fresh. Both had precipitated abundant deposit. Solution C was clear. For the P_H measurement (quinhydrone) this solution was diluted with 3 volumes of water.

III. STABILISATION EXPERIMENTS.

In working with enzyme solutions their slight stability is a great drawback. The proteinase is, sufficiently stable in raw malt extracts, whereas the peptidase is rather quickly broken up. As previously mentioned the experiments also seem to indicate that an addition of toluene to the extract had a favourable effect on the stability of the peptidase. This I have found in certain cases, in others not. In all cases the dialysed water malt extracts are much more unstable than the undialysed ones, and the peptidase is rapidly destroyed with toluene as well as without it. The stability of the proteinase is also somewhat reduced owing to the precipitation, when the solutions are left to stand, of a viscous sediment, difficult to suspend in the solution and containing part of the proteinase. Even if the destruction of the peptidase is partly due to an acidity which increases on standing

owing to bacterial infection, and which makes itself strongly felt in these liquids poor in buffer, yet this is not the only factor that is important for the stability (table 11), and a simple addition of toluene is not sufficient to prevent inactivation of the peptidase. I therefore made a change and made dialysed malt extracts stable with glycerine, adding to them the same volume of this (III G, IV G etc.). As shown in the following tables, which need no further explanation, the stability, especially of the peptidase, is greatly increased by this.

IV. SEPARATION OF PEPTIDASE AND PROTEINASE.

A. Introduction.

The experiments dealt with in this main section must be regarded as examples taken at random from my experimental data. I have, especially with aluminium hydroxide, conducted a number of adsorption experiments, which did not yield particularly reproducible results, but which all showed the incomplete separation of proteinase and peptidase by means of this adsorption agent. I have considered it necessary to exemplify more copiously only in case of the favourable ferric hydroxide method. I emphasise that the experiments were planned solely for the purpose of finding a practical method of separation, not with a theoretical study of the adsorption curves in view. They must be regarded in this light.

The following examples may serve as an explanation of the general technic of the experiments :

Exp. 1. Adsorption experiment with aluminium hydroxide.

5 cc of malt extract III G, containing 153 LG-U and 160 Ed-U see table 7', were mixed with 1.0 cc of acetic acid- sodium acetate buffer of P_H 4.7, 3.5 cc of water and 0.5 cc of $Al(OH)_3$. Total volume 10 cc, glycerine conc. in the mixture 22 %. It was centrifuged, and the residual solution (theoretically 10 cc generally a little less) was examined for peptidase and proteinase, being diluted with 1/2 volume of water before the determinations. Per 5 cc of digestion liquid was added 2 cc of diluted residual solution corresponding to c_E 0.8 or to c_E 0.533 of undiluted residual solution, which is again equivalent to 0.4 cc of undialysed malt extract III '0.533 $V_b/4V_a = 0.4$). The glycerine concentration in the digestion liquids was 5.9 %, and

the x -values found, $x_{LG}=0.47$ and $x_{Ed}=0.28$, were corrected by multiplication with the factors 1.12, 1.13 respectively, read from the curves fig. 1 and 3: x_{LG} corr. = 0.53, corresponding to 4.2 LG-U in 0.533 cc of residual solution or 79 LG-U in 10 cc, yield 79. 100/153 = 52 %: x_{Ed} corr. = 0.32, corresponding to 3.9 Ed-U in 0.533 cc of residual solution or 73 per 10 cc, yield 73. 100/160 = 46 %.

Exp. 2. Elution experiment with ferric hydroxide adsorbate.

Elution agent primary phosphate.

2.5 cc of VG, containing 116 LG-U and 141 Ed-U (cf. table 7) were mixed with 3 cc of water, 2 cc of n 100 ammonia (making P_H 8.4) and 2.5 cc of ferric hydroxide. Total volume 10 cc. The glycerine concentration in the mixture was 11 %. It was centrifuged. The residual solution contained only traces of enzymes. The deposit was stirred up with the following mixture: 7.5 cc of m 15 primary potassium phosphate + 1.25 cc of glycerine + 1.25 cc of water \rightarrow 10 cc (glycerine concentration 11 %). It was again centrifuged and the eluate (10 cc) was investigated for proteinase and peptidase; after addition of 1 n ammonia to P_H 8, as far as the latter determination was concerned. c_E was 0.8; the glycerine concentration in the digestion liquid 4.4 %; the phosphate concentration m /50. I found: $x_{Ed}=0.09$, corrected for glycerine error 0.10 (phosphate correction 0 see p. 28) corresponding to 1.2 Ed-U in 0.8 cc or 15 Ed-U in all; yield 11 %. $x_{LG}=0.15$ which served for the calculation of the yield in the following manner: (see p. 30). First I measured the cleavage of leucylglycine by the malt extract with and without phosphate:

a. Without phosphate, $c_E=0.2$; $x_{LG}\sim 1.05$.

b. m /50 with respect to phosphate buffer of P_H 8, $c_E=0.2$; $x_{LG}=0.40$, according to which the yield of the peptidase was computed as follows:

0.15. 1.05. 100/0.4. 1.05 = 38%, the $c_E=0.8$ of the eluate corresponding to $c_E=0.2$ for malt extract VG.

All the x -values stated in the following tables are those directly found, without correction for glycerine- or phosphate-errors.

B. Adsorption Experiments with Aluminium Hydroxide.

The results of the experiments stated in table 12 are as follows: throughout the P_H -interval examined, the proteinase is more freely adsorbed than the peptidase. The displacement obtained in the proportion between the enzymes is greatest at neutral or slightly alkaline reaction—in contrast to the conditions in yeast—but is not sufficient to encourage further experiments. The preparation and age of the aluminium hydroxide does not seem to play any considerable part, as the little experiment with malt extract IVG may serve to exemplify. This experiment also represents the best separation I have obtained with aluminium hydroxide.

TABLE 12.

Adsorption of proteinase and peptidase by $\text{Al}(\text{OH})_3$, $\text{C}_\gamma(\text{Al})$ at varying P_H .

In each experiment 5 cc of malt extract III G in 10 cc of adsorption mixture corresponding to a total of 153 LG-U and 160 Ed-U. Glycerine concentration in the adsorption mixture 22%. At the determination of enzyme $c_E = 0.533$. Glycerine concentration 5.9.

P_H	Composition of the adsorption mixture per 10 cc			Substrate	Enzyme content in residual solution. Volume 10 cc		
	cc n/100 ammonia	cc acetate-buffer 1:1 1 n.	cc $\text{Al}(\text{OH})_3$ & C_γ Al_2		\times	Enzyme units total	Yield %
4.7	0	1.0	0.5	Leucylglycine	0.47	79	52
6.4	0	0	"		0.65	107	70
7	2.0	0	"		0.80	135	88
8	3.1	0	"		0.77	126	83
4.7	0	1.0	"	Edestin	0.28	73	46
6.4	0	0	"		0.36	98	61
7	2.0	0	"		0.39	105	66
8	3.1	0	"		0.34	90	56
7	2.0	0	0.5	Leucylglycine	0.80	135	88
"	"	"	1.0		0.42	69	45
"	"	"	1.25		0.23	39	26
"	"	"	0.5	Edestin	0.39	105	66
"	"	"	1.0		0.17	43	27
"	"	"	1.25		0.07	17	11

TABLE 13.

Adsorption of proteinase and peptidase by 2 different kinds of $\text{Al}(\text{OH})_3$, Al_1 and Al_2 at P_H ca. 8.

In each experiment 2.5 cc of malt extract IV G and 1.25 cc of $\text{Al}(\text{OH})_3$ in 10 cc of adsorption mixture, corresponding to a total of 115 LG-U and 133 Ed-U. Glycerine concentration in the adsorption mixture 11%. At the enzyme determination $c_E = 0.8$. Glycerine concentration 4.4%.

P_H	Comp. of adsorption mixture per 10 cc cc n/100 ammonia	Kind of $\text{Al}(\text{OH})_3$	Substrate	Enzyme content in resid. sol. Vol. 10 cc		
				\times	Enzyme units total	Yield %
8	2.00	Al_1	Leucylgl.	0.10	0.11	9
"	"	"	Edestin	0.02	0	0
"	"	Al_2	Leucylgl.	0.18	0.19	17
"	"	"	Edestin	0.03	0	0

A clear idea of the adsorption conditions is easily gained from fig. 5.

Fig. 5.

- A. Enzyme yield in residual solution from adsorption with 0.5 cc $\text{Al}(\text{OH})_3$ per 10 cc adsorption mixture at various P_H
- B. Enzyme yield in residual solution from adsorption with various quantities of $\text{Al}(\text{OH})_3$ at P_H 7.

(See Table 12, first part).

(See Table 12, last part).

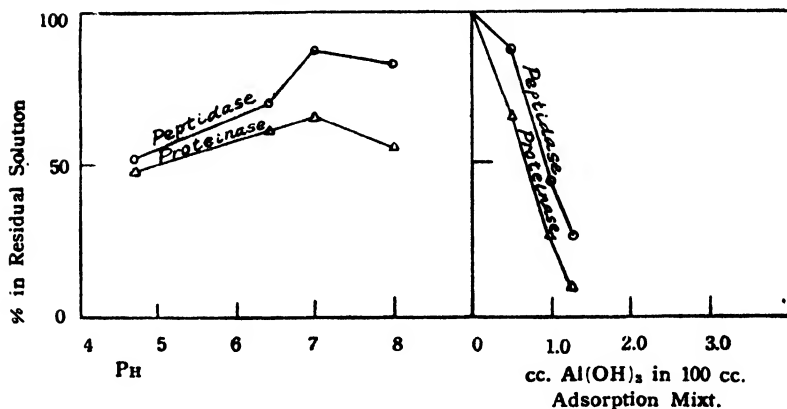
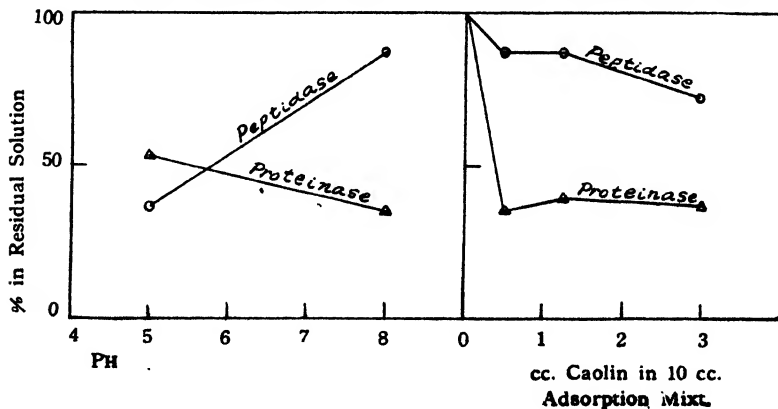


Fig. 6.

- A. Enzyme yield in residual solution from adsorption with 0.5 cc caolin per 10 cc adsorption mixture at various P_H .
- B. Enzyme yield in residual solution from adsorption with various quantities of caolin at P_H 8.

(See Table 14, upper part).

(See Table 14, lower part).



C. Adsorption Experiments with Caolin.

The experiments in table 14, graphically illustrated in fig. 6, show the experiments made with caolin. As with aluminium hydroxide the separation is best at slightly alkaline reaction where the adsorption of the proteinase is the higher in proportion to that of the peptidase. But at this P_H the increase of the caolin amount does not cause any increase in adsorption, so that caoline is hardly suitable for quantitative separation of these enzymes. I expressly emphasise that repeated adsorption offers no advantage.

TABLE 14.

Adsorption of proteinase and peptidase by caolin at varying P_H . In each experiment 2.5cc of malt extract IVG in 10cc adsorption mixture, corresponding to a total of 115 LG-U and 133 Ed-U. Glycerine concentration in the adsorption mixture 11 %. At the enzyme determination c_E 0.8. Glycerine concentration 4.4 %.

P_H	Composition of adsorption mixt. per 10 cc			Substrate	Enzyme content in resid. sol. Vol. 10 cc		
	cc n 100 ammonia	cc n 100 acetic acid	cc caolin			Enzyme units total	Yield %
5	0	0.75	0.5	Leucyl-glycine	0.41	43	37
8	2.0	0	"		0.93	100	87
5	0	0.75	"	Edestin	0.40	70	53
8	2.0	0	"		0.28	48	36
8	2.0	0	0.5	Leucylglycine	0.93	100	87
'	"	"	1.25		0.93	100	87
'	"	"	3.0		0.78	83	72
'	"	"	0.5	Edestin	0.28	48	36
'	"	"	1.25		0.31	53	40
"	"	"	3.0		0.29	49	37

D. Adsorption Experiments with Caolin and Aluminium Hydroxide.

By combined adsorption with caolin and aluminium hydroxide at P_H ca. 8, a fairly good separation as shown in table 15 was obtained. The method was, however, dropped for the ferric hydroxide method.

TABLE 15.

Adsorption of proteinase and peptidase with caolin and $Al(OH)_3$, Al_2 , successively, at P_H ca. 8.

1. 2.5 cc IVG, 115 LG-U and 133 Ed-U in all, +1.48 cc n/100 ammonia +1.25 cc caolin \rightarrow 10 cc, centrifuged. To the residual solution added 0.52 cc n/100 ammonia and 0.6 cc $Al(OH)_3$, when it was again centrifuged. 10 cc residual solution. At the determination $c_E=0.8$, glycerine concentration ca. 4.4 %.

2. Like 1, only with 0.9 cc $Al(OH)_3$.

No. of exp.	Substrate	\times	Enzyme units in resid. sol. total	% of original
1	Leucylglycine	0.65	69	60
	Edestin	0.11	18	14
2	Leucylglycine	0.31	34	30
	Edestin	0.04	0	0

E. Adsorption Experiments with Ferric Hydroxide.

As shown in tables 16 and 17, and in fig. 7, the adsorption of peptidase and proteinase with $Fe(OH)_3$ generally proceeds in the same way as the aluminium hydroxide and caolin adsorption. The enzyme separation is least favourable in acid solution, best at P_H 8.4 under application of 1.5 cc of $Fe(OH)_3$ in 10 cc of adsorption mixture. Here the proteinase content of the residual solution drops to zero, while the peptidase content is still 40% of the original content before the adsorption. With larger amounts of ferric hydroxide both peptidase and proteinase are entirely adsorbed. At P_H 7, 1.5 cc of $Fe(OH)_3$ is sufficient for the complete adsorption of both enzymes.

This adsorption method is the most favourable one investigated and I have therefore adopted it for the preparation of proteinase-free

peptidase solutions, with the modification of increasing the glycerine concentration in the adsorption mixture. This partly caused an increased yield of peptidase, and partly tended to make it more stable after the adsorption, (cf. p. 50). The experiment in table 18 shows the influence of the glycerine on the adsorption. It was made with malt extract VG 10 days after those described in tables 16 and 17, to which fact the somewhat smaller yield may be ascribed.

Table 19 contains yet another example of a ferric hydroxide adsorption with malt extract VIIG, where the yield of peptidase is particularly good. Here conditions are somewhat different, 2.0 cc and not 1.5 cc Fe(OH)₃ per 10 cc adsorption mixture being necessary for

TABLE 16.

Adsorption of proteinase and peptidase with Fe(OH)₃ at varying P_H.
In each experiment 2.5 cc of malt extract IVG for 10 cc adsorption mixture, corresponding to a total of 115 LG-U and 133 Ed-U. The glycerine concentration in the adsorption mixture 11%. C_E=0.8 at the enzyme determination. Glycerine concentration 4.4%.

P _H	Composition of adsorption mixture per 10 cc			Substrate	Enzyme yield in residual sol. 10 cc		
	cc n 100 ammonia	cc n 100 acetic acid	cc Fe-OH ₃		×	Enzyme units total	% of original
4.7	0	0.75	0.5	Leucyl-glycine	0.38	40	35
8.4	2.0	0	0.5		0.83	89	77
4.7	0	0.75	0.5	Edestin	0.23	38	29
8.4	2.0	0	0.5		0.23	38	29

In each experiment 2.5 cc of malt extract VG in 10 cc of adsorption mixture, corresponding to a total of 116 LG-U and 141 Ed-U. Glycerine in the adsorption mixture 11%. C_E=0.8 at the enzyme determination. Glycerine concentration 4.4%.

7.0	0.9	0	1.5	Leucyl-glycine	0.06	6	5
8.4	2.0	0	1.5		0.15	48	41
7.0	0.9	0	1.5	Edestin	0.04	0	0
8.4	2.0	0	1.5		0.01	0	0

complete adsorption of the proteinase at P_H 8.4. Altogether it cannot be expected that the different malt extracts should yield absolutely the same quantitative results under the same conditions. Individual differences (age etc.) which must be taken into account assert themselves, so that in any given case test must be made for the removal of the proteinase. This does not, however, detract from the general applicability of the method.

TABLE 17.

Adsorption of proteinase and peptidase with varying amounts of $Fe(OH)_3$ at $P_H=8.4$.

In each experiment 2.5 cc of malt extract IVG or VG in 10 cc of adsorption mixture, corresponding to a total of 115 LG-U and 133 Ed-U, or 116 LG-U and 141 Ed-U respectively. Glycerine concentration in the adsorption mixture 11 %. At the enzyme determination $c_E=0.8$. Glycerine concentration 4.4 %.

P_H	Malt extract	Composition of adsorption mixture per 10 cc		Substrate	Enzyme yield in residual sol. 10 cc		
		cc n/100 ammonia	cc $Fe(OH)_3$		x	Enzyme units total	% of original
8.4	IVG	2.0	0.5	Leucylglycine	0.83	88	77
"	VG	"	1.5		0.45	46	40
"	"	"	"		0.43	44	38
"	"	"	"		0.44	45	39
"	"	"	"		0.45	46	40
"	"	"	2.5		0.04	5	4
8.4	IVG	2.0	0.5	Edestin	0.23	38	29
"	VG	"	1.5		0.04	0	0
"	"	"	"		0.02	0	0
"	"	"	"		0.04	0	0
"	"	"	"		0.01	0	0
"	"	"	2.5		0.02	0	0

Fig. 7.

A. Enzyme yield in residual solution from adsorption with 0.5 and 1.5 cc $\text{Fe}(\text{OH})_3$ per 10 cc adsorption mixture at various P_H .

B. Enzyme yield in residual solution from adsorption with various quantities of $\text{Fe}(\text{OH})_3$ at P_H 8.4.

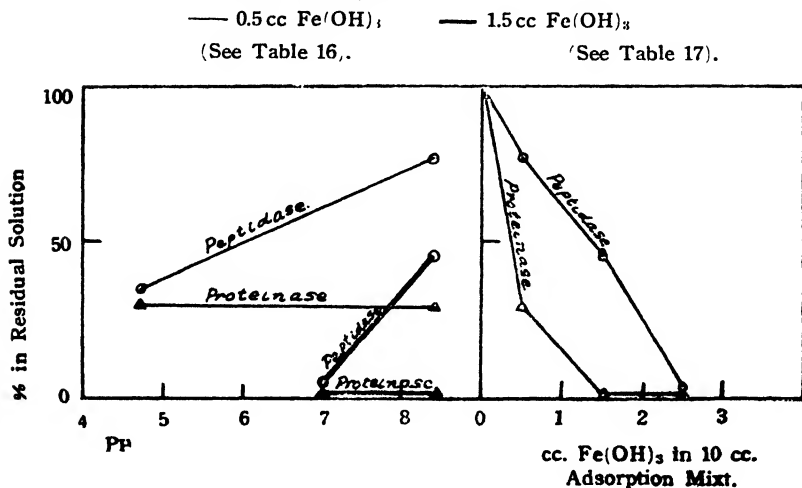


TABLE 18.

The influence of glycerine on the ferric hydroxide adsorption.

- 2.5 cc of malt extract VG, total 116 LG-U and 141 Ed-U + 2 cc n/100 ammonia + 1.5 cc $\text{Fe}(\text{OH})_3 \rightarrow 10$ cc. P_H 8.4, Glycerine concentration 11 %. At the determination $c_\text{E} = 0.8$; Glycerine concentration 4.4 %.
- Like 1, only per 10 cc added 3.75 cc of 88 % glycerine. Glycerine concentration 17.6 %.

Experiment No.	Substrate	x	Enzyme yield in residual solution. 10 cc	
			Enzyme units total	% of original
1	Leucylglycine	0.33	35	30
	Edestin	0.04	0	0
2	Leucylglycine	0.36	50	43
	Edestin	0.04	0	0

TABLE 19.

Adsorption experiment with malt extract VIIG, ferric hydroxide.

1. 5 cc of malt extract VIIG, total 192 LG-U and 185 Ed-U +5 cc glycerine +2.25 cc $\text{Fe}(\text{OH})_3$ +2.7 cc n/100 ammonia \rightarrow 15 cc, P_H ca. 8.4; glycerine concentration in adsorption mixture 39 %. At the determination c_E^- 0.8. Glycerine concentration 17.6 %.
2. Like 1, only with 4.3 cc of glycerine and 3.0 cc $\text{Fe}(\text{OH})_3$. Glycerine concentration in adsorption mixture 39 %. At the determination c_E^- 0.8. Glycerine concentration 15.6 %.

Experiment No.	Substrate	x	Enzyme yield in residual solution. 15 cc	
			Enzyme units total	% of original
1	Leucylglycine	0.78	163	85
	Edestin	0.15	51	28
2	Leucylglycine	0.49	98	51
	Edestin	0.03	0	0

F. Elution of Peptidase and Proteinase from the Ferric Hydroxide Adsorbates.

The reason why I have made elution experiments in so very few cases is that an elution agent like ammonia in most cases gives but poor results while phosphates, which are excellent elution agents, owing to their inhibition of the peptidase, must as far as possible be avoided. In the series of main experiments with ferric hydroxide, I have, however, thought it necessary to show that the existing displacement of the proportion between peptidase and proteinase is not mainly due to a partial destruction of the enzymes, but that these can really, at least with a tolerable yield be dissolved from the connection with the adsorption agent.

Tables 20 and 21, which require no further explanation (for the method of computation see p. 30 and the example p. 40), provide the adequate proof. It is interesting that even primary phosphate eludes tolerably well and that secondary phosphate is a poorer eluant than phosphate of P_H 6.8, which latter fact is in accordance with the adsorption experiments in table 16 and fig. 7 A. This is well

TABLE 20

Elution of ferric hydroxide adsorbates with phosphates at varying P_H . Three adsorption mixtures of following composition were prepared; 2.5 cc of malt extract VG, total 116 LG-U and 141 Ed U + 2 cc n/100 ammonia + 2.5 cc $Fe(OH)_3 \rightarrow 10$ cc; P_H 8.4. Glycerine concentration 11 %. They were centrifuged. The residual solutions contained only traces of enzymes (cf. table 17). The precipitates were eluted with following 3 solutions:

1. 7.5 cc m/15 primary potassium phosphate + 1.25 cc 88 % glycerine
2. 7.5 cc m/15 phosphate buffer of P_H 6.8 + 1.25 cc 88 % glycerine
3. 7.5 cc m/15 secondary sodium phosphate + 1.25 cc 88 % glycerine

Glycerine concentration 4.4 %.

Experiment No.	Substrate	x	Enzyme yield in eludates Volume 10 cc	
			Enzyme units total	% of original
1	Leucylglycine	0.15	—	38
	Edestin	0.09	15	11
2	Leucylglycine	0.20	—	50
	Edestin	0.25	45	32
3	Leucylglycine	0.23	—	58
	Edestin	0.10	16	11

TABLE 21.

Elution of ferric hydroxide adsorbates with ammonia. 2.5 cc of malt extract VG, total 116 LG-U and 141 Ed U, was mixed with n 100 ammonia, 1.5 cc $Fe(OH)_3$ and water, to 10 cc. P_H 7.2. Glycerine concentration 11 %. It was centrifuged. The residual solution contained only traces of enzymes (cf. table 16). The precipitate was eluted with the following solution:

4 cc n 100 ammonia + 1.25 cc glycerine \rightarrow 10 cc. Glycerine concentration 11 %. At the determinations, $c_F = 0.8$. Glycerine concentration 4.4 %. The experiment was repeated, 1 and 2.

Experiment No.	Substrate	x	Enzyme yield in the eludates. Volume 10 cc	
			Enzyme units total	% of original
1	Leucylglycine	0.21	23	20
	Edestin	0.05	0	0
2	Leucylglycine	0.20	21	18
	Edestin	0.05	0	0

shown, too, in the ammonia elution experiment (table 21), where at the adsorption at P_H 7. I have both peptidase and proteinase completely adsorbed: Elution at higher P_H draws out the peptidase without perceptibly influencing the proteinase, which is firmly attached to the adsorption agent, also in basic solution.

G. Methods of Separation of Proteolytic Enzymes.

The preceding experiments have shown the proportion of peptidase and proteinase in malt extracts may conveniently be changed. On the basis of the experience gained, the following preparation methods can be recommended:

Proteinase solutions free from peptidase are prepared by letting the dialysed water malt extract stand, at 1° for a long time; at higher temperature with toluene for a short time. (cf. the example of malt extract IV, p. 37).

TABLE 22.

The stability of glycerine containing residual solution after the removal of proteinase by $Fe(OH)_3$. P_H ca. 7. Left to stand at 1° with toluene.

obtained from malt extract	Days of standing	LG-cleavage x
VG	0	0.36
"	4	0.36
"	7	0.35
VIIG	0	0.48
"	10	0.51

Peptidase solutions free from proteinase are prepared by adsorption with ferric hydroxide in 44% glycerine at P_H ca. 8. The proteinase-free residual solution is neutralised, toluene is added, and the solution is stored at 1° . As shown in table 22, the stability of the solution thus obtained was pretty good.

SUMMARY.

This paper deals with the behaviour of malt-proteases, and stating the methods used for their determination and separation.

1) At least two proteases, one peptidase and one proteinase are present in malt. The activity of the peptidase was measured by its cleavage of leucylglycine at P_{II} 8 (p. 28), that of the proteinase by its cleavage of edestin at P_{II} 4.1 (p. 23). In the case of both enzymes the increase of carboxyl groups during a fixed time of digestion at 40° was determined by titration in alcohol.

2) The peptidase is unstable in water solution, but it may be stabilised by an addition of glycerine (p. 38).

3) The best way of separating the peptidase from the proteinase is by adsorption with ferric hydroxide at P_{II} 8, glycerine being added. Up to 50% of the peptidase will remain in the proteinase-free residual solution by this method. Peptidase-free proteinase is most easily obtained by leaving malt water extracts to stand, thus decomposing the peptidase (pp. 44 and 48).

After a completed proof-reading of the Danish edition of this paper, a paper on malt proteases by H. Lüers and L. Malsch^{1a)} has appeared in *Wochenschrift für Brauerei*. Broadly speaking my adsorption experiments with $Al(OH)_3$ confirm those conducted by these authors. But their method of separation of these two proteolytic enzymes seems not to be better than my method owing to the reason that they used the extract without dialysis for the adsorption experiments and consequently, phosphates, etc., contained in the extract might have exercised an influence eludingly against the adsorption of both the enzymes, thus, making the complete separation in a good yield rather difficult.

(B)

ON THE PEPTIDASES OF GREEN MALT.

THEORETICAL PART

In a previous publication the author¹⁵⁾ has described some investigations of the cleavage of GG (glycylglycine), AG (alanylglycine) and LG (leucylglycine) by means of malt peptidases. In these investigations, I found such large variations in the relation between the velocities with which these substances were split up by different enzyme-preparations, that I concluded provisionally that in extracts of green malt (just as in intestinal extracts) there are two peptidases, of

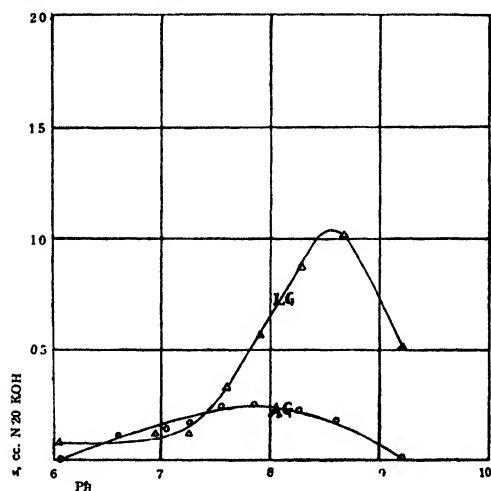


Fig. I.

Cleavage of LG and AG with proteinase-free malt extract 7 D (see pp. 62-64) at varying P_H . The LG-cleavage is principally due to peptidase II, optimum at P_H 8.6, and the AG-cleavage principally to peptidase I, optimum at P_H 7.8. Substrate conc. (c_{AG} , c_{LG}) 0.1 mol. 2 hours' cleavage at 40° . $c_E = 0.2$. c_E = number of cc. enzyme solution per 2 cc of digest. (See further Table IV, p. 67 and p. 62). \times = increase in number of carboxyl groups, expressed in cc KOH per 2 cc. digestion liquid.

which the one—peptidase I—having its P_H -optimum at 7.8, principally splits up AG, while the other—peptidase II—having its P_H -optimum at 8.6, principally splits up LG (cf. Tables IV and V and Figures I and II in the present work). I found peptidase II in a comparatively pure state in certain dialysed aqueous malt extracts, particularly those which had been freed from the proteinase of malt by adsorption with ferric hydroxide¹⁶⁾ while I found both enzymes, though principally peptidase I, in a glycerine extract of malt. EULER,

MYRBÄCK and MYRBÄCK⁴⁾ published almost simultaneously with the above-mentioned paper¹⁶⁾ experimental results which are in agreement with mine.

The present work constitutes a continuation of the previous investigations, and has as its essential aim the elucidation and determination of the affinity of the peptidase-complex of green malt for the peptides AG and LG. It was hoped thereby to obtain, if possible, support for the hypothesis previously advanced. It was found by GRASSMANN and KLENK¹¹⁾ that the affinity of the peptidase-complexes of the kidneys and of yeast is much less for GG than for LG. In fact, while the velocity of LG-cleavage (v_{LG}) is for these enzymes practically independent of the substrate concentration, the velocity of GG-cleavage (v_{GG}) increases rapidly with the GG concentration, which according to well known theories (MICHAELIS, KUHN) can be interpreted in the above-mentioned manner. They find for different enzyme-samples variations in the ratio $Q_{GG+LG} = v_{GG}/v_{LG}$ similar to the variations found by LINDERSTRÖM-LANG and the author. This they consider to be most simply explained by assuming that their enzyme-samples contain only one enzyme, a dipeptidase, but that there are also present varying amounts of certain hypothetical inhibitors which combine with this enzyme to give inactive compounds, from which leucylglycine can

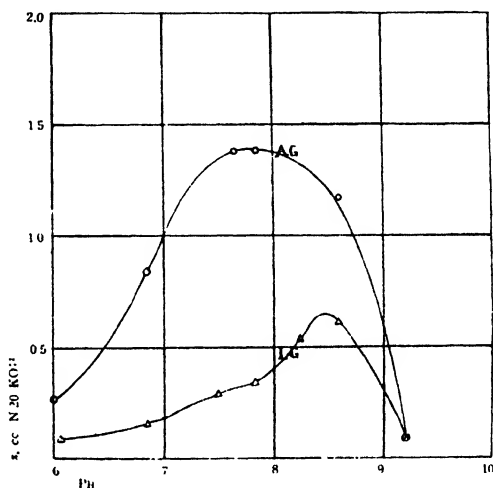


Fig. II.

Cleavage of LG and AG with fresh glycerine malt extract 8 'see pp. 62-64'. The LG-cleavage is due partly to peptidase I and partly to peptidase II; the AG-cleavage principally to peptidase I. $c_E - 0.1$, time of cleavage 1 hr. Otherwise the same as Fig. I. (Cf. also Table V, p. 68 and p. 62).

the velocity of GG-cleavage (v_{GG}) increases rapidly with the GG concentration, which according to well known theories (MICHAELIS, KUHN) can be interpreted in the above-mentioned manner. They find for different enzyme-samples variations in the ratio $Q_{GG+LG} = v_{GG}/v_{LG}$ similar to the variations found by LINDERSTRÖM-LANG and the author. This they consider to be most simply explained by assuming that their enzyme-samples contain only one enzyme, a dipeptidase, but that there are also present varying amounts of certain hypothetical inhibitors which combine with this enzyme to give inactive compounds, from which leucylglycine can

under all circumstances completely liberate the enzyme, while glycylglycine on account of its small enzyme-affinity can only bring about an incomplete liberation, which will be less complete the greater the amount of inhibitor in the enzyme-sample. However, the actual affinity relations in the case of the malt-enzymes differ substantially from this scheme. Thus EULER, MYRBÄCK and MYRBÄCK find that GG inhibits to a great extent the cleavage of LG by malt-extract of low GG cleaving activity, which goes to show that in this case the affinity of the dipeptidase for GG is at all events not less than for LG. Nothing is as yet known as regards the AG-affinity.

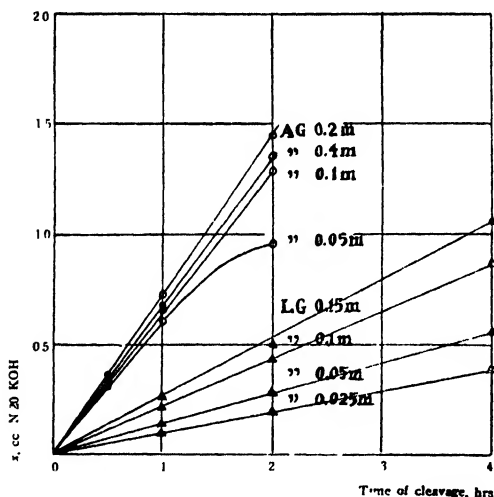


Fig. III.

Cleavage of LG and AG with aqueous extract 20 (pp. 62-64., $Q_1 = \frac{x_{AG}}{x_{LG}} - 3.0$)

for $c_{AG} - c_{LG} = 0.1$ mol. Varying substrate concentration, $P_H = 7.9$. Enzyme conc. corresponding to 0.034 g green malt per 2 cc digest. Cleavage at 40° . For further details of symbols see Fig. 1, Tables VII and X, (pp. 69 and 70) and p. 62.

MANN and KLENK.

In Figs. III and IV are shown graphically the results of two typical affinity determinations, the first (Fig. III) carried out with a

In a footnote to their paper Grassmann and Klenk call attention to the fact that for the enzymes studied by them, the affinity for AG is also smaller than that for LG, and on the grounds of their hypothesis propose this fact as a basis for explaining the variations in the quotient $Q_{AG/LG} = v_{AG}/v_{LG}$ found by the author. However, the investigations which are described in the following pages give a picture of the facts which is entirely different, and in some cases the reverse of that proposed and by GRASS-

malt-extract having a Q_1 value of 3.0, and the second (Fig. IV) with a malt-extract having a Q_1 value 40 times as small, i. e. 0.078. Q_1 is defined as the ratio x_{AG}/x_{LG} , where x_{AG} and x_{LG} are the number of carboxyl groups (expressed in cc of $n/20$ KOH per 2 cc digest) formed in the same time by the splitting up of two peptides AG and LG respectively under the same experimental conditions. If these figures are to be explained on the basis of MICHAELIS and KUHN's ideas and GRASSMANN

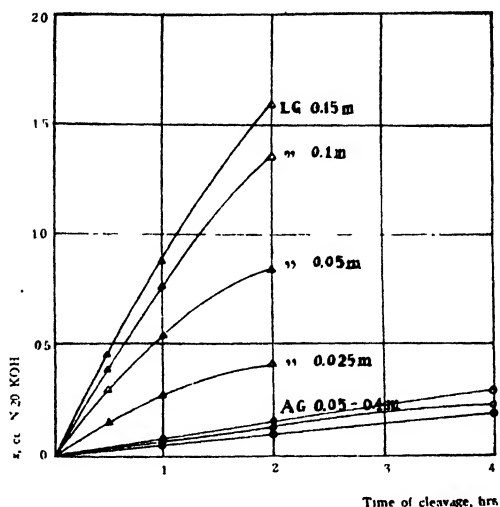


Fig. IV.

Cleavage of LG and AG with glycerine extract 21 pp. 62-64', Q_1 -0.078 for $c_{AG} \cdot c_{LG} = 0.1$ mol. Varying substrate concentration, $P_H = 7.9$. Enzyme concentration corresponding to 0.058 g green malt per 2 cc digest. Cleavage at 40° . For further details of symbols see Figs. 1 and III, Tables VIII and XI pp. 69 and 71 and p. 62.

and KLENK's hypothesis, we are forced to the conclusion that the dipeptidase in malt has a greater affinity for AG than for LG, and that in the case of the first peptide, the combination of the enzyme with the substrate is practically complete for all the AG-concentrations studied—i. e., the opposite conclusion to that arrived at GRASSMANN and KLENK. At the P_H employed in these experiments (7.9) it was impossible on account of the small solubility of LG to increase the LG concentration further in order to determine at what concentration of substrate the combination of LG and enzyme becomes complete, and it is doubtful whether it is possible in this way to obtain results susceptible of theoretical treatment. At higher P_H , where the solubility of LG is greater, and the concentration of substrate can therefore be increased up to 0.4 mol, I find (as will be further described in the experimental section p. 65) that the velocity of cleavage does

not assume a stationary value, but first increases with increasing LG-concentration, reaches a maximum at about 0.1–0.2 mol, and then decreases again. The cause of this cannot yet be stated with certainty, but it is possible that the slightest impurities in the substrate play some part. I have also found a similar outcome in the velocity of cleavage at high substrate concentrations in the case of AG-cleavage (c_{AG} above 0.4 mol). (cf. also GRASSMANN and KLENK¹¹⁾).

On account of the large enzyme-affinity of alanylglycine and the incomplete determination of the curve for leucylglycine, these experiments are not suited for the calculation of the ratio of the enzyme-affinities of the two peptides. However, by assuming GRASSMANN and KLENK's interpretation, it is possible to obtain some idea of this ratio, e. g. by investigating the inhibitory action of added LG upon the cleavage of AG¹⁴⁾, or, in other words, by determining the distribution of the dipeptidase between the two peptides. For an experiment of this kind, it is necessary to use an enzyme solution with a high Q-value, so that the LG-cleavage is small in comparison to the AG-cleavage. Fig. V shows graphically an experiment of this nature. It

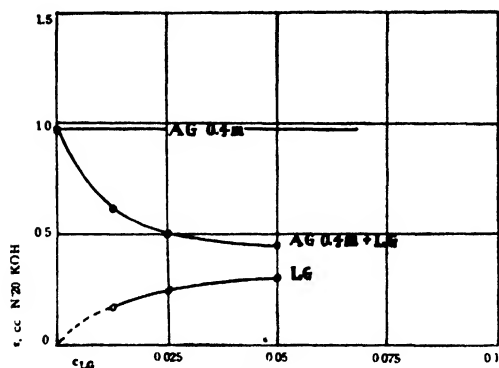


Fig. V.

Inhibition of AG-cleavage by the addition of LG in varying concentrations. Malt extract 19, pp. 62–64 Q_1 –2.6. Time of cleavage 1 hour. c_E –0.1, P_H –7.9. For further details of symbols see the previous figures and p. 74. The curves correspond to the figures in Table XV. (p 62).

shows in a striking manner the strong inhibiting action of leucylglycine, which is most simply explained by assuming that it has the greater affinity for the dipeptidase, though this assumption is in complete contradiction to the results of the experiments shown in Figs. III and IV. An approximate calculation of the ratio of the affinities (cf. ¹⁴⁾) gives the result that the LG-affinity

is about 32 times as great as the AG-affinity, assuming that both cleavages are due to the same enzyme.

Therefore I find it impossible to see how this contradiction can be removed except by the assumption that the cleavage is due to the action of two enzymes.

In parallel with the leucylglycine cleavage, the leucylglycylglycine cleavage was also followed during the stability - as well as during the adsorption - experiments. Here the ratio between the rates at which the two peptides are hydrolysed has proved the same in all cases, viz. ca. 1. In contrast to the conditions in yeast,^{78,9)} where the polypeptidase keeps pace with the proteinase, the tripeptide cleaving power here keeps pace with the dipeptide cleaving power; they are decomposed with equal rapidity (a characteristic difference from the enzymes of yeast), and the ratio between them is not displaced by adsorption. These experiments alone render it probable that we are here dealing with two effects of the same enzyme, and this view is supported by the determination of the P_H -curves of the two enzymatic powers, these curves coinciding so completely that there is hardly any reason for ascribing a different enzyme to each of them. (Fig. X).

Experiments have also been carried out on the splitting up of LGG (leucylglycylglycine), AGG (alanylglycylglycine) and LGGG (leucyldiglycylglycine). These experiments show that even fresh glycerine-extract of green malt, which gives rise to a vigorous splitting up of LGG (with optimum at P_H 8.6) is only able to attack AGG to a small degree, and that the small cleavage of this peptide found seems to have its P_H -optimum at 7.8. LGGG is on the other hand vigorously split up, with the maximum in the neighbourhood of P_H 8.6. It cannot yet be stated with certainty whether this cleavage is due to peptidase II (cf. ¹⁶⁾) or another enzyme, but the observation is of considerable interest in connection with the fact previously discovered¹⁶⁾, that the cleavage of both LG and LGG is due to peptidase II. (See p. 75).

The experiments described in the present paper were carried out in essentially the same way as previously stated¹⁶⁾. There have

however been introduced several improvements for increasing the accuracy of the enzyme-determinations, which is particularly necessary with high substrate concentrations. This has been effected partly by the use of the double digestion vessels and partly by recognition of the fact that the use of rubber stoppers for closing the digestion vessels constitutes a serious source of error, and the consequent use of cork stoppers instead (See also p. 60 for more detailed improvements in technique).

EXPERIMENTAL PART

I. METHODS OF PREPARATION

A. Preparation of Enzyme-Solutions.

1. *Extracts Prepared from Fresh Green Malt.*

a) Undialysed glycerine extracts.

Pulverized green malt was extracted with an equal volume of concentrated glycerine and filtered at room temperature. The filtrate was collected every day, and brought to -10° , at which temperature it was stored. The glycerine content of these extracts was estimated as 60%, allowing for the water content of the green malt.

b) Dialysed glycerine extracts.

The glycerine extracts obtained by the above method were dialysed under reduced pressure against 44% glycerine, and were after dialysis diluted to the volumes they had before dialysis with glycerine of the same concentration.

c) Undialysed aqueous extracts.

These were prepared by extracting pulverized green malt for 2 hours at 30° with $\frac{1}{5}$ its volume of water, and subsequently filtering through a Buchner funnel.

d) Dialysed aqueous extracts.

The crude aqueous extracts were dialysed as previously described and after dialysis were stabilised by the addition of an equal volume of 88% glycerine.

e) Proteinase-free peptidase solution.

The dialysed aqueous extract (with the addition of glycerine) was adsorbed with ferric hydroxide as previously described¹⁶⁾. The remaining solution was proteinase-free.

2. Extracts Prepared from Dried Green Malt.

a) Dried green malt.

The method for the preparation of dried green malt was essentially that employed by Willstätter and Waldschmidt-Leitz¹⁷⁾ for drying pancreas.

1800 g of pulverized green malt was treated 4 times with 2 litres of acetone, then twice with a mixture of 1 litre of acetone and 1 litre ether, and finally twice with 2 litres of pure ether. Each operation was performed rapidly, and the suspensions were filtered immediately without standing. The product thus obtained was dried between filter papers and sifted through a sieve with 1 mm meshes. The yield was 778 g of finely sifted powder and 237 g of a crude product consisting chiefly of husks, etc. Since the crude product was low in enzyme content compared with the sifted powder, only the latter was used in preparing the following extracts.

b) Aqueous extract of dried green malt.

20 g of dried green malt were well mixed with 100 cc of water in a mortar, and the liquid filtered as well as possible through a Buchner funnel. The filtrate thus obtained was centrifuged for 15 min. in order to clarify it further, mixed with an equal volume of concentrated glycerine, and kept standing at +1°.

c) Glycerine extract of dried green malt for affinity determinations.

20 g of dried green malt were well mixed with 100 cc of concentrated glycerine, 0.5 cc of toluene added, and the mixture was kept in a bottle with a cork stopper standing for 36 hours at 40°. 100 cc of water was then added, and the liquid filtered and centrifuged in the same manner as for the aqueous extract. It was stored at +1°.

B. Preparation of Substrates.

All substrates were racemic. They were prepared according to FISCHER's method¹⁵⁾ and analysed for carboxyl-groups, amino-groups and total nitrogen. The peptides were recrystallized at least once. The substrate buffer solutions were carefully filtered.

C. Preparation of Substrate Solutions.

This preparation has been previously¹⁶⁾ described and no essential alterations have been made. P_{H} was adjusted and measured in the same way as before. The concentration of the buffer-solution was the same. The substrate concentration was varied by mixing concentrated substrate buffer solutions with buffer solutions having the same P_{H} , and the P_{H} of the mixtures was carefully checked. Ammonia-ammonium chloride buffers were used throughout.

II. ANALYTICAL METHOD

A. Method of Determination.

The digestion experiments were carried out on the same lines as before¹⁶⁾, but as stated in the introduction, the method was improved in some respects*, partly by the use of cork stoppers in place of rubber ones, and partly by the introduction of the double digestion vessels shown in Fig. VI. The total volume of the vessels used was 11 cc. for the ordinary determinations, and about 21 cc for the affinity determinations, in which the cleavage was to be investigated at different time-intervals.

Fig. VI.



The experiments were carried out in the following manner. The substrate buffer solution was pipetted into one limb, and the enzyme solution (diluted with glycerine, water etc.) into the other. The volumes of the two solutions were identical—2.5 cc. for ordinary deter-

* Exception must be made in the case of experiments in tables IV, V, XVI, XVII, XVIII, XIX, and XX which are taken from earlier work, and were carried out as described there 16).

minations, and 5 cc. for affinity determinations. The vessel was put in a thermostat at 40°, and warmed for 10 minutes. It is important that the vessel should be immersed in the thermostat right up to the upper part of the neck, both during warming and during the digestion itself, as otherwise liquid condenses round the stopper, which gives rise to errors in the determination. After warming, the enzyme and substrate solutions were mixed by rapidly shaking the vessel, and 2 cc. of the mixture were taken out (as previously described) before and after the period of digestion. The reaction was stopped with 30 cc. of 96% alcohol. In pipetting out the sample, the pipette was dried externally with a piece of filter paper, held with its tip against the wall of the titration-flask, and after the liquid had run out, it was carefully blown out twice. The digestion temperature was throughout 40°.

Titration was carried out with N/20 KOH, as previously described¹⁶⁾.

In Table I is given a typical experiment showing the effect of new rubber stoppers on the splitting up of alanylglycine. With rubber stoppers which have been used once or twice the error is not nearly so great as it is here, but the danger that inhibiting substances will be introduced is by no means negligible, and cork stoppers are to be preferred. It is also an advantage to avoid as far as possible letting the digestion liquid come in contact with the stopper during mixing and pipetting.

TABLE I.

Comparison of cleavage experiments with new rubber stoppers and cork stoppers. Experiments carried out in a test-tube according to the earlier method. The liquid came much into contact with the stopper while mixing the enzyme and substrate. Malt extract 15. $c_E = 0.05$. P_H 7.9. $c_{AG} = 0.1$. Glycerine concentration 10%. 2 hours' cleavage at 40°.

	x_{AG}
Rubber stoppers	0.74 0.94
Cork stoppers (same experiment)	1.32 1.31

B. Symbols.

For the sake of reference I have collected here the symbols used in the preceding and following sections.

t = time of cleavage.

c_E = enzyme concentration expressed in cc of enzyme solution per 2 cc of digestion mixture or enzyme concentration reduced to grams of dried green malt used to prepare that amount of malt extract used per 2 cc of digestion mixture.

c_{AG} , c_{LG} , etc. = substrate concentration expressed in mol.

x_{AG} , x_{LG} , etc. = number of carboxyl groups formed by the splitting up of the peptide, expressed in cc. of N/20 KOH per 2 cc. digestion mixture.

$Q_I = x_{AG}/x_{LG}$, when $c_{AG} = c_{LG} = 0.1$ mol, $P_{II} = 7.9$ and the other experimental conditions are the same for the cleavage of both peptides.

$Q_s = x_{AG}/x_{LG}$, when $c_{AG} = c_{LG} = 0.1$ mol, $P_{II} = 7.9$ for alanylglycine cleavage and 8.5 for leucylglycine cleavage. The remaining experimental conditions are the same for the cleavage of the two peptides.

Tadle II a.
Survey of the crude malt extracts used.

Number of malt extract	Date of preparation	Number of days during which the extract was collected	Nature of malt extract	
7	17/5 1929	1	From fresh malt	Aqueous "
8	11/6	5		Glycerine "
12	12/10	5		" "
15	21 11	5 - 10		" "
16	31/1 1930	1		Aqueous "
17	"	7	From dried malt	Glycerine "
18	14/6	1		Aqueous "
19	27/6	3		Glycerine "
20	24/11	-		Aqueous "
21	"	-		Glycerine "

III. MALT EXTRACTS EMPLOYED.

Tables IIa and IIb contain a survey of the malt extracts used in the investigations, and the particular data of their preparation. They were all prepared from Danish brewery malt, malted for 9 days.

TABLE IIb.
Survey of the dialysed malt extracts used.

	7 D	16 D	17 D	18 E
Time of standing, days.	1	1	8	1
Duration of dialysis, days.	4	7	20	5
Ratio of volumes before and after dialysis	3:1	20:7	38:15	5:1
P _H before dialysis.....	6.0	5.9	5.7	6.05
P _H after dialysis.	6.0	5.8	6.3	5.82
Dialysed against.	water	water	44 % glycerine	water

The malt extracts 7—12 were prepared from malt from April 1929, extracts 15—17 from malt from November 1929, extracts 18 and 19 from malt from May 1930, and finally extracts 20 and 21

TABLE IIIa.
The Q_1 values of the malt extracts. C_G - % glycerine
in the digestion mixture.

Enzyme solution	C_E	C_G	t hours	X_{AG}	X_{LG}	Q_1
7 D	0.2	8	2	0.25	0.59	0.45
8	"	3	1	1.38	0.36	3.8
15	0.04	10.4	1	0.63	0.24	2.6
"	"	"	2	1.17	0.46	2.5
19	0.1	15	1	1.14	0.44	2.6
20	*	"	1	0.65	0.22	3.0
21	†	"	1	0.06	0.77	0.078

* corresponding to 0.034 g. dry green malt

† " " 0.068 g. " " "

from malt from October 2nd 1930. All the samples of malt were kept standing at -10° until the date of preparation.

Tables IIIa and IIIb contain various determinations of the Q values of these extracts.

For the comparison between the power of malt extracts to digest leucylglycine and their power to digest leucylglycylglycine were used malt extracts I, II, VIIG, etc., (see pp. 33-35) owing to the reason that these experiments were carried out during the work of separation of the proteolytic enzymes.

TABLE IIIb.

The Q-values of the malt extracts. C_G —% glycerine in the digestion mixture.

Enzyme solution	c_E	c_G	t hours	x_{AG}	x_{LG}	Q:
7 D	0.2	8	2	0.25	1.02	0.25
8	0.1	3	1	1.38	0.61	2.3
12	0.2	6	2	1.25	0.74	1.7
15	0.2	6	1	*1.87	1.16	1.6
"	0.1	12.6	1	1.29	0.69	1.9
16 D	0.2	10.4	2	0.14	1.04	0.13
17	0.04	"	"	0.83	0.48	1.7
"	0.1	"	"	1.41	0.86	1.6
17 D	"	"	"	1.04	0.87	1.2

* almost complete cleavage.

IV. EXPERIMENTAL RESULTS ON THE AFFINITY EXPERIMENTS.

The experimental results are given in the following tables and curves. No further comment is necessary, except with regard to one point, the calculation of the ratio of the affinities of the dipeptidase

for LG and AG, from the results of the experiments in Table XIV or XV. I shall here consider those in Table XV.

The calculation is carried out on the basis of the equations:

$$v_{AG+LG} = 1/2 (v_{2AG} + v_{2LG}) \quad (1)$$

and

$$\frac{c_{AG}}{c_{LG}} = \frac{k_{AG}}{k_{LG}} \quad (2)$$

v_{2AG} and v_{2LG} signify the initial velocities of cleavage of the two peptides when present separately at concentrations $2c_{AG}$ and $2c_{LG}$ respectively, while v_{AG+LG} is the initial velocity of the total splitting up when a mixture containing the two peptides at concentrations c_{AG} and c_{LG} is submitted to the action of the enzyme. As has been shown previously¹⁴⁾ the validity of equation (1) requires that the ratio of c_{AG} to c_{LG} is that given by equation (2), i. e., the ratio of the affinity constants for the enzyme-substrate compounds of the two peptides. On account of the linear nature of the curves connecting cleavage with time, v_{AG} and v_{LG} , etc. can be replaced by the values of x_{AG} and x_{LG} , etc., obtained after, say, one hour's reaction. Further, since in the concentration range 0.05—0.4, x_{AG} is very nearly independent of c_{AG} (see Tables VI and VII), we can for an approximate calculation of this kind also replace x_{2AG} by x_{AG} , though it must be noticed that we are thereby ignoring the fact that x_{AG} falls at higher values of c_{AG} (see bottom of Table XV), a fact which cannot be explained by the hypothesis upon which equations (1) and (2) are based. Equation (1) thus becomes

$$x_{AG+LG} = 1/2 (x_{AG} + x_{2LG}).$$

It is seen from Table XV that this relation is fulfilled when $c_{AG}=0.4$, $c_{LG}=0.0125$, $2c_{LG}=0.025$, so that

$$\frac{k_{AG}}{k_{LG}} = \frac{0.4}{0.0125} = 32.$$

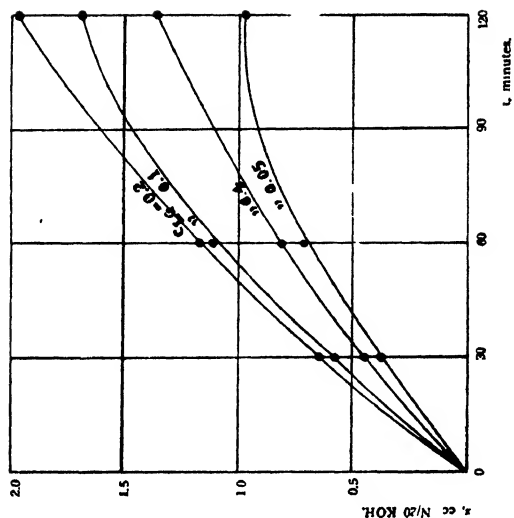


Fig. VII.

Cleavage of LG and AG with malt extract 15 pp. 62-64. $Q_1 = 2.6$. Varying substrate concentration, $P_H 7.9$. Glycerine conc. 10.4%. $c_g = 0.04$. The data corresponding to the curves are given in Tables VI and IX pp. 68 and 70. For details of symbols see p. 62.

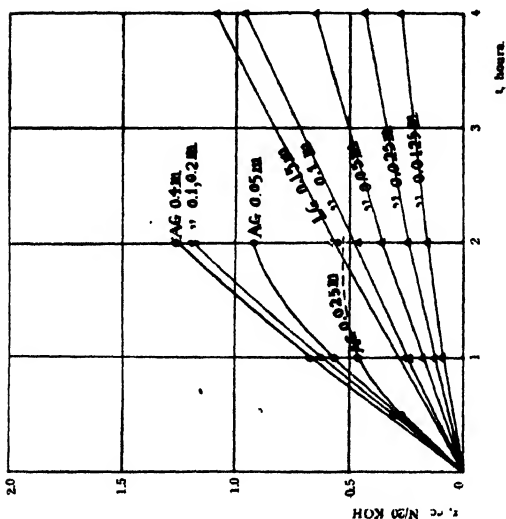


Fig. VIII.

Cleavage of LG with aqueous extract 18 D. Varying substrate conc. $c_g = 0.4$. Glycerine conc. 15%. $P_H 8.5$. The curves correspond to the data in Table XIII, p. 72.

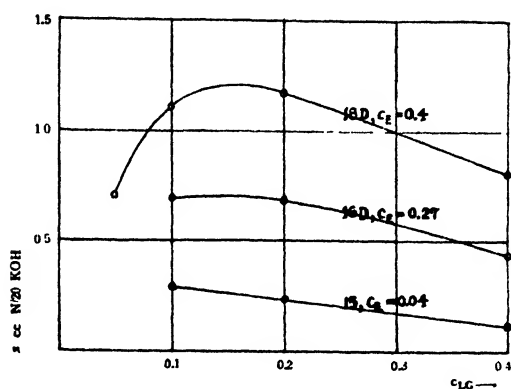


Fig. IX.

Cleavage of LG with different values of c_{LG} and various enzyme preparations. $t=60$ minutes. $P_H=8.5$. The curves correspond to the figures in Tables XII and XIII, pp. 71 and 72.

TABLE IV.

Cleavage of alanylglycine and leucylglycine with proteinase-free malt extract from malt extract 7 D. $C_E=0.2$. Substrate conc. 0.1 mol. Glycerine conc. 8 %. 2 hours' cleavage at 40°. Cf. Fig. I.

Alanylglycine				Leucylglycine			
P_H			\times	P_H			\times
before and after cleavage				before and after cleavage			
ca.	6.05	-	0	ca.	6.05	-	0.09
„	6.6	-	0.11	„	6.6	-	0.09
	7.05	7.05	0.14	„	6.95	-	0.12
	7.25	7.25	0.17		7.25	7.25	0.12
	7.55	7.65	0.24		7.6	7.65	0.33
	7.85	7.95	0.25		7.9	7.95	0.56
	8.25	-	0.22		8.3	8.35	0.87
	8.6	-	0.18		8.65	8.65	1.02
	9.2	-	0.01		9.2	9.2	0.51

TABLE V.

Cleavage of alanylglycine and leucylglycine with malt extract 8. $C_E=0.10$. Substrate conc. 0.1 mol. Glycerine conc. 3 %. 1 hour's cleavage at 40°. Cf. Fig. II.

Alanylglycine			Leucylglycine		
P_H before and after cleavage		\times	P_H before and after cleavage		\times
6.0	6.0	0.27	6.05	6.05	0.09
6.85	6.85	0.84	6.85	6.85	0.16
7.65	7.85	1.38	7.5	7.5	0.29
7.85	8.05	1.38	7.85	7.85	0.35
8.6	8.6	1.17	ca. 8.25	—	0.54
9.2	9.2	0.09	8.6	8.6	0.61
—	—	—	9.2	9.2	0.09

TABLE VI.

Cleavage of alanylglycine at P_H 7.9 with various substrate concentrations. Glycerine malt extract 15. $C_E=0.04$. t =time of digestion in minutes. Glycerine conc. ca. 10.4 %.

C_{AG}	\times_{AG}					
	t=30	Mean	t=60	Mean	t=120	Mean
0.4	0.27	0.29	0.70	0.67	1.24	1.26
"	0.30		0.64		1.27	
0.2	0.27	0.28	0.66	0.63	1.16	1.19
"	0.29		0.60		1.22	
0.1	0.31	0.30	0.64	0.63	1.15	1.17
"	0.29		0.62		1.19	
0.05	0.27	0.28	0.56	0.56	0.93	0.92
"	0.28		0.56		0.91	
0.025	0.27	0.26	0.46	0.46	0.54	0.55
"	0.24		0.46		0.56	

TABLE VII.

Cleavage of alanylglycine at P_H 7.9 with various substrate concentrations. Aqueous extract 20 of dried green malt. C_E corresponds to 0.034 g. dried green malt. t =time of digestion in minutes. Glycerine conc. of digestion mixture 15 %.

C_{AG}	x_{AG}					
	t=30	Mean	t=60	Mean	t=120	Mean
0.4	0.32	0.32	0.64	0.65	1.36	1.36
"	0.31		0.66		1.36	
0.2	0.36	0.37	0.74	0.73	1.45	1.45
"	0.38		0.72		1.44	
0.1	0.33	0.35	0.67	0.68	1.29	1.29
"	0.36		0.69		1.29	
0.05	0.33	0.33	0.60	0.61	0.96	0.96
"	0.33		0.62		0.96	

TABLE VIII.

Cleavage of alanylglycine at P_H 7.9 with various substrate concentrations. Glycerine extract 21 of dried green malt, allowed to stand at 40° for 36 hours. C_E corresponds to 0.068 g. dried green malt. t time of digestion in hours. Glycerine conc. of digestion mixture 15 %.

C_{AG}	x_{AG}					
	t=1	Mean	t=2	Mean	t=4	Mean
0.4	0.07	0.07	0.12	0.14	0.26	0.28
"	0.07		0.15		0.29	
0.2	0.07	0.07	0.13	0.13	0.28	0.28
"	0.07		0.13		0.28	
0.1	0.06	0.06	0.12	0.12	0.22	0.22
"	0.06		0.11		0.21	
0.05	0.05	0.05	0.09	0.09	0.18	0.18
"	0.05		0.09		0.18	

TABLE IX.

Cleavage of leucylglycine at P_H 7.9 with various substrate concentrations. Glycerine malt extract 15. $c_E \approx 0.04$. Glycerine conc. 10.4 %. t =time of digestion in minutes.

c_{LG}	x_{LG}		
	$t=60$	$t=120$	$t=240$
0.15	0.24	0.58	1.09
0.10	0.24	0.46	0.97
0.05	0.18	0.36	0.64
0.025	0.13	0.25	0.44
0.0125	0.11	0.15	0.28

TABLE X.

Cleavage of leucylglycine at P_H 7.9 with various substrate concentrations. Aqueous extract 20 of dried green malt. c_E corresponds to 0.034g dried green malt. t =time of digestion in hours. Glycerine conc. 15 %.

c_{LG}	x_{LG}					
	$t=1$	Mean	$t=2$	Mean	$t=4$	Mean
0.15	0.26	0.27	0.50	0.51	1.07	1.07
"	0.27		0.52		1.07	
0.10	0.23	0.22	0.44	0.44	0.89	0.88
"	0.21		0.43		0.87	
0.05	0.14	0.15	0.29	0.29	0.58	0.57
"	0.15		0.29		0.56	
0.025	0.11	0.11	0.20	0.20	0.39	0.39
"	0.11		0.20		0.39	

TABLE XI.

Cleavage of leucylglycine at P_H 7.9 with various substrate concentrations. Glycerine extract 21 of dried green malt, kept standing at 40° for 36 hours. c_E corresponds to 0.068 g. dried green malt. t =time of digestion in minutes. Glycerine concentration of digestion mixture 15 %.

c_{LG}	x_{LG}					
	t=30	Mean	t=60	Mean	t=120	Mean
0.15	0.46	0.46	0.88	0.88	1.60	1.59
"	—		0.88		1.58	
0.10	0.38	0.39	0.76	0.77	1.34	1.35
"	0.39		0.78		1.35	
0.05	0.28	0.30	0.55	0.55	0.84	0.84
"	0.31		0.54		0.84	
0.025	0.16	0.15	0.28	0.27	0.43	0.42
"	0.14		0.26		0.41	

TABLE XII.

Cleavage of leucylglycine at P_H 8.5 with various substrate concentrations. Glycerine malt extract 15 c_E 0.04. Aqueous malt extract 16 D C_E 0.27 Glycerine conc. 10.4 %. t =time of digestion in minutes.

Malt extract	c_{LG}	x_{LG}					
		t=60	Mean	t=120	Mean	t=240	Mean
15	0.4	0.13	0.12	0.17	0.16	0.35	0.35
"		0.10		0.14		0.35	
	0.2	0.24	0.24	0.44	0.46	0.75	0.75
"		0.24		0.48		0.76	
"		0.23		0.45		0.75	
	0.1	0.30	0.29	0.56	0.55	0.99	0.98
"		0.29		0.56		0.98	
"		0.29		0.54		0.96	

16 D		t=30	Mean	t=60	Mean	t=120	Mean
	0.4	0.24	0.23	0.45	0.44	0.79	0.78
	"	0.21		0.42		0.77	
	0.2	0.31	0.32	0.67	0.68	1.23	1.24
	"	0.32		0.68		1.25	
	0.1	0.30	0.30	0.69	0.69	1.25	1.25
	"	0.30		0.68		1.25	

TABLE XIII.

Cleavage of leucylglycine at P_H 8.5 with various substrate concentrations. Aqueous malt extract 18 D. $c_E=0.4$. Glycerine conc. 15 %. t =time of digestion in minutes.

c_{LG}	x_{LG}					
	t=30	Mean	t=60	Mean	t=120	Mean
0.4	0.40	0.44	0.79	0.80	1.44	1.37
"	0.46		0.88		-	
"	0.45		0.79		1.39	
"	0.49		0.87			
"	0.43		0.72		1.32	
"	0.41		0.84		1.36	
"	0.45		0.72		1.32	
"	0.40		0.76		-	
0.2	0.64	0.64	1.21	1.17	2.06	1.97
"	0.62		1.17		2.03	
"	0.66		1.16		1.88	
"	0.63		1.15		1.92	
"	0.64		1.16		1.95	
0.1	0.57	0.58	1.11	1.11	1.70	1.69
"	0.57		1.11		1.67	
"	0.57		1.10		1.70	
"	0.59		1.10		1.67	
"	0.59		1.11		1.70	
"	0.58		1.11		1.71	
"	0.59		1.10		1.68	
0.05	0.36	0.37	0.71	0.71	-	0.97
"	0.38		0.73		0.97	
"	0.37		0.71		-	
"	0.38		0.70		0.99	
"	0.36		0.69		0.96	

TABLE XIV.

Cleavage of alanylglycine with the addition of varying concentrations of leucylglycine. Malt extract 15. P_H 7.9. $c_E = 0.04$. Glycerine conc. 10.4% One hour's digestion at 40°.

c_{AG}	c_{LG}	$\times AG + LG$	
		Found	Mean
0.4	0.05	0.29	0.29
"	"	0.28	
0.1	0.025	0.34	0.36
"	"	0.38	
0.4	0.0125	0.38	0.38
0.4	0	0.60	0.60
"	0	0.60	
0	0.05	0.16	0.17
"	"	0.17	
"	"	0.17	
"	"	0.19	
0	0.025	0.12	0.12
"	"	0.12	
0	0.0125	0.10	0.10

TABLE XV.

Cleavage of alanylglycine with the addition of varying concentrations of leucylglycine. Glycerine malt extract 19, undialysed. $c_E=0.1$. P_H 7.9. Glycerine concentration 15%. One hour's digestion at 40° .

c_{AG}	c_{LG}	$\Sigma AG+LG$	
		Found	Mean
0.4	0.05	0.44	0.45
		0.44	
		0.46	
		0.44	
		0.47	
0.4	0.025	0.52	0.50
		0.50	
		0.49	
		0.50	
0.4	0.0125	0.58	0.61
		0.61	
		0.61	
		0.62	
0.4	0	1.02	0.98
		0.92	
		0.93	
		0.97	
		1.02	
0	0.05	1.04	0.30
		0.29	
		0.29	
		0.29	
		0.31	
		0.31	
0	0.025	0.32	0.24
		0.24	
		0.23	
		0.24	
0	0.0125	0.23	0.17
		0.17	
		0.16	
		0.18	
0.8	0	0.17	0.56
		0.50	
		0.50	
		0.57	
		0.54	
		0.62	
		0.60	

V. EXPERIMENTAL RESULTS ON THE COMPARISON BETWEEN THE POWER OF MALT EXTRACTS TO DIGEST LEUCYLGLYCINE AND THEIR POWER TO DIGEST LEUCYLGLYCYLGLYCINE.

As mentioned in the theoretical part, it might be expected, according to GRASSMANN's fundamental researches on the proteases of yeast that in malt extracts, just as in autolysates of yeast, a polypeptidase was present which differed from the enzyme acting upon the dipeptide leucylglycine. In a number of cases I have therefore investigated their power to split up the tripeptide leucylglycylglycine

TABLE XVI.

The relation between the amount of enzyme and the cleavage of leucylglycine and leucylglycylglycine. Malt extract I diluted with 3 volumes of water before the determination. P_H 8.0. Substrate conc. 0.1 mol. Glycerine conc. 0.1 hour's cleavage at 40°.

c_E	x_{LG}	x_{LGG}	x_{LG}/x_{LGG}
0.2	0.58	0.66	0.88
0.4	1.16	1.21	0.96
0.8	1.69	1.75	0.96

TABLE XVII.

The decrease in the leucylglycine- and leucylglycylglycine cleaving power of malt extracts I and II when left to stand. P_H 8.0. Substrate conc. 0.1 mol. Glycerine conc. 0.1 hour's cleavage at 40°.

Kind of enzyme	Days of standing	c_E	x_{LG}	x_{LGG}	x_{LG}/x_{LGG}
Malt extract I stored at 1° without dilution	1	0.1	1.16	1.21	0.96
	23	"	0.07	0.06	1.16
Malt extract II, like I.	1	0.1	1.17	1.13	1.04
	5	"	0.91	0.97	0.94
	9	"	0.75	0.72	1.04
Malt extract II diluted 1:4 after standing 5 days in undiluted state. Standing at 1°.	0	0.4	0.91	0.97	0.94
	4	"	0.16	0.14	1.14

(LGG) at the same time as I have followed the LG-cleavage. The method is given on pp. 28 and the malt extracts used on p. 33-35.

Table XVI shows the relation between the enzyme concentration and LG- and LGG-cleavage. The experiment was conducted with the dialysed malt extract I diluted with three volumes of water immediately before the investigation. As is shown, the x-values proceed quite in parallel.

TABLE XVIII.

Comparison of the leucylglycine- and the leucylglycylglycine cleaving power of malt extract VIIG and of its residual solutions from adsorption experiments with caolin and ferric hydroxide.

1. 5 cc. VIIG + 4.3 cc. glycerine + 2.7 cc. n/100 ammonia + 3 cc. ferric hydroxide → 15 cc. Centrifuged. Residual solution 1. $P_H=8$.
2. 5 cc. VIIG + 5.0 cc. glycerine + 2.7 cc. n/100 ammonia + 1 cc. caolin → 15 cc. Centrifuged. Residual solution 2. $P_H=8$.

Substrate conc. 0.1 mol. 1 hour's cleavage at 40°. The stated x-values are those directly found, without correction for glycerine error.

Kind of enzyme solution	c_E	x_{LG}	x_{LGG}	x_{LG}/x_{LGG}
Malt extract VIIG	0.267	1.06	1.08	0.98
Resid. sol. 1 [Fe(OH)]	0.8	0.52	0.55	0.95
Resid. sol. 2 [Caolin]	0.8	0.87	0.93	0.94

TABLE XIX.

Cleavage of leucylglycine with proteinase-free peptidase solution at varying P_H . Substrate conc. 0.1 mol. $c_E=0.4$. Glycerine concentration 8.8%. Time of digestion 2 hours. 40°.

P_H before cleavage	P_H after cleavage	Increase cc. n/20 KOH
ca. 6	—	0.09
„ 6.6	—	0.09
„ 7	—	0.12
7.25	7.25	0.12
7.60	7.65	0.33
7.90	7.95	0.56
8.30	8.35	0.87
8.65	8.65	1.02
9.20	9.20	0.51

Table XVII shows the parallel decrease of the rate of hydrolysis of both substrates, which occurs on the malt extracts being left to stand. From table XVIII it is likewise seen that x_{LO}/x_{LOG} retains the same value also after the adsorption with caolin as well as with ferric hydroxide. These examples show that there is hardly any reason to suppose that we are here dealing with two enzymes.

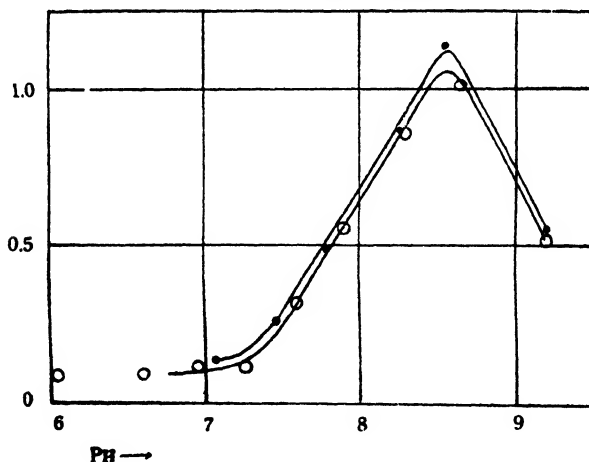
The experiments made on the P_{II} optimum both of the leucylglycine- and the leucylglycylglycine-cleavage are conclusive. The experiments were made with a proteinase-free peptidase solution, prepared as described on p.50. P_{II} was adjusted by help of acetic acid and ammonia, the substrate-buffer-stock solution (p. 28) forming the starting point, and with a few exceptions (the three lowest P_{II} values

Fig. X.

PH-Optimum for the malt peptidase.

○ Cleavage of leucylglycine.

● Cleavage of leucylglycylglycine.



(See Tables XIX and XX, pp. 76 and 78).

in table XIX, where the approximate P_{II} of the substrate-buffer-mixtures before the enzyme addition is stated) P_{II} was measured in solutions which were prepared identically with the actual digestion liquids, immediately and after the time of digestion. The results are shown in tables XIX and XX and in figure X.

It appears from this that there is no perceptible difference in the P_H optimum, and the curves lie so close together that both actions must undoubtedly be presumed to be caused by the same enzyme.

TABLE XX.

Cleavage of leucylglycylglycine with proteinase-free peptidase solution at varying P_H . Substrate conc. 0.1 mol. $c_E=0.4$. Glycerine concentration 8.8 %. Time of digestion 2 hours. 19'.

P_H before cleavage	P_H after cleavage	Increase cc. n/20 KOH
7.05	7.05	0.14
7.45	7.45	0.26
7.80	7.85	0.48
8.25	8.30	0.87
8.55	8.55	1.12
9.20	9.20	0.55

VI. EXPERIMENTAL RESULTS ON THE POWER OF MALT EXTRACTS TO DIGEST ALANYLGLYCYLGLYCINE AND LEUCYLDIGLYCYLGLYCINE.

The experimental results are given in the following tables. No further comment is necessary, except that they were obtained by the method described on p. 60.

TABLE XXI.

Cleavage of LG, LGG, AG and AGG. Malt extract 12. $c_E=0.2$. P_H 7.9 for x_{AG} , x_{AGG} and 8.5 for x_{LG} and x_{LGG} . Substrate conc. 0.1 mol. Glycerine conc. 6 %. 2 hours' cleavage at 40'.

x_{LG}	x_{LGG}	x_{AG}	x_{AGG}
0.80	1.10	1.35	0.15

TABLE XXII.

Cleavage of AGG at different P_H values. Malt extract 12. $c_E=0.2$. Substrate conc. 0.1 mol Glycerine conc. 6%. 2 hours' cleavage at 40°.

P_H approx.	x_{AGG}
6.05	0.12
6.95	0.12
8.0	0.16
8.0	0.13
8.4	0.12
8.4	0.12

TABLE XXIII.

Cleavage of LGGG at different P_H values. Malt extract 15. $c_E=0.2$. c_{LGGG} 0.1 mol. Glycerine conc. 6%. 1 hour's cleavage at 40°.

P_H approx.	x_{LGGG}
8.0	0.50
8.5	0.68
9.1	0.59

SUMMARY B.

The affinity of the peptidase complex of malt for the peptides alanylglycine (AG) and leucylglycine (LG) has been determined. The malt extracts employed had a power of cleavage which was in some cases high for AG and low for LG (Nos. 15, 19 and 20, see pp.62-64) and in other cases low for AG and high for LG (Nos. 16 D and 21, pp.62-64). For the different enzyme solutions, Q (the ratio between the powers of cleavage for AG and LG) varied from 3.0 to 0.078.

In the affinity-determinations, chief importance was allotted to investigating the manner in which the velocity with which the peptide was split up depended on the substrate concentration. It was found :

1. At P_H 7.9 the velocity of AG-cleavage was only to a small extent dependent on the substrate concentration when the latter was less than about 0.4 mol. This was true no matter what the Q value of the malt extract used. Over about 0.4 mol it was found in a single instance that the velocity of cleavage decreased rapidly with increasing substrate-concentration (Table XV p. 74). On the other hand, the velocity of LG-cleavage always increases rapidly with increasing substrate-concentration in the range 0.0125—0.15. With the experimental method used here it was not possible to increase the LG-concentration further at this P_H owing to the low solubility of this peptide.

2. At P_H 8.5 the velocity of LG-cleavage increases with substrate concentration until the latter has reached a value between 0.1 and 0.2, after which it decreases again with increasing substrate concentration. (Fig. IX. p. 67).

As a check on these determinations some experiments were carried out on the inhibition of AG-cleavage by the addition of LG. A strong inhibitory effect was found. (Tables XIV and XV, Fig. V).

On the basis of these experiments (which are dealt with more fully in the introduction) it is very difficult to maintain the view that only one enzyme is effective in splitting up these peptides. For the

time being the simplest explanation is that two or more enzymes in malt have a dipeptidase character.

The cleavages of LG and LGG are quite similar during the stability- as well as adsorption experiments. The P_H -optimum of peptidase lies at 8.5–8.6, no matter whether the dipeptide leucylglycine or the tripeptide leucylglycylglycine be used as substrate. According to these results, there is hardly any reason for ascribing a different enzyme to each cleavage of these two peptides. (Fig. X. p. 77).

Fresh glycerine extracts of malt, which split up LG, LGG, LGGG and AG powerfully, only have a small power of attacking AGG. (Tables XXI, XXII, and XXIII).

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ENZYME CHEMICAL INVESTIGATION OF FORMOSAN SNAKE VENOMS.

I. On the Proteolytic Enzymes in the Venom of Taiwan-habu, (*Trimeresurus mucrosquamatus*, CANTOR).§

(With 11 Text-Figures)

Masakazu SATO and Tamotu HIRANO.

(Accepted for publication, October 23, 1935)

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§ One part of this paper was reported⁽⁵⁾ already at the 10th meeting of the Japanese Association for Advancement of Science, held in December 1934 at Taihoku in Formosa.

[Mem. of the Fac. of Sci. and Agr., Taikoku Imp. Univ., Formosa, Japan, Vol. IX, No. 3, October, 1935].

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INTRODUCTION

On the snake venoms, a great many investigations have been carried out from the point of view of serology, while a very few investigations have been done from the standpoint of enzyme chemistry. However, according to our opinion, thorough investigation in the line of the latter should reveal a great many significant fresh factors which are full of much interest. Therefore, the authors undertook an enzyme chemical investigation of Formosan snake venoms on a somewhat large scale. In the present paper is given an investigation of the proteolytic enzymes in the venom of Taiwan-habu.

Investigators such as MITCHELL and REICHERT, FLEXNER and NOGUCHI, LAUNOY, CALMETT and NOC⁽²⁾ have reported that snake venoms contain the substances which denature muscle tissue, fibrin and other proteid substances. In Formosa, YAMAGUCHI⁽⁷⁾ and SENBON⁽⁶⁾ have also demonstrated that fibrin is disintegrated by snake venoms. However, these investigations quoted above were all mainly due to only the physical changes and not at all to the chemical changes of the substrate.

Therefore we established at first the most suitable chemical method

for the determination of proteolytic enzymes and then tested the enzymatic power of the venom with substrates such as casein, gelatin, eggalbumin, haemoglobin and Witte's peptone, etc. The results obtained are summarized on page 95.

It is our pleasant duty to express our cordial thanks to the Imperial Academy, for the subsidy we received for our researches.

EXPERIMENTAL PART

I. METHODS OF PREPARATION.

A. Preparation of Enzyme Solutions.

1. *Preparation of Dried Snake Venom.*

The fresh snake venom which is viscous and light yellow colored liquid was dried in a desiccator with CaCl_2 under reduced pressure. The powdered dry venom ground in an agate mortar was left to stand before use in a desiccator.

2. *Preparation of Venom Solution.*

1% or 0.5% venom glycerine (30%) solution or water solution was prepared, and in both cases the solution was centrifuged and then filtered. This cleared solution was employed as enzyme solution.

1% or 0.5% venom glycerine solution was used for the test of the splitting of all substrates except that of haemoglobin where water venom solution was used owing to the reason stated on the foot note of Table 2.

Each venom solution was prepared just before every digestion experiment.

B. Preparation of Substrate Solutions.

1. Substrate Solutions Prepared.

The survey of the substrate solutions employed is given in the following table.

TABLE 1.
Survey of substrate solutions prepared.

No.	Substrate solutions	pH
1	6% casein-NaOH solution	7.0 (at 20°)
2	6% casein-pH 8.0 buffer solution	8.0 (at 40°)
3	6% gelatin solution	5.4 (at 20°)
4	3% eggalbumin solution	6.1 (")
5	3% haemoglobin solution	8.1 (")
6	3% Witte's peptone solution	7.1 (")

2. Procedure of Preparation.

6% casein solution: 6 g of casein (after Hammarsten, Merck) were weighed in a 100 cc measuring flask and were suspended in small quantities of water and then mixed with 36 cc of $n/10$ NaOH. After complete dissolution the flask was filled up with water to the mark.

6% casein-pH 8.0 buffer solution: 6 g of casein (after Hammarsten, Merck) were mixed with ca. 25 cc of water and 23 cc of $n/5$ NaOH. After complete dissolution 40 cc of $n/1$ NH_4Cl - NH_4OH (8:1) buffer were introduced and the mixture was diluted with water to 100 cc.

6% gelatin solution: 6 g of gelatin (Gold-label, Kahlbaum) were dissolved in small quantities of water warming at ca. 50° and diluted with water to 100 cc after it cooled to ca. 35°.

3% haemoglobin solution: 1.5 g of haemoglobin (Merck) were ground in an agate mortar with the addition of water and transferred

into a 50 cc measuring flask. The mixture was warmed at 40° for 10 minutes and centrifuged. The supernatant dark red colored solution was employed.

3% eggalbumin as well as 3% Witte's peptone: 3 g of eggalbumin (Merck) or 3 g of Witte's peptone (Grübler) were dissolved in 100 cc of water and filtered.

II. ANALYTICAL METHODS

A. Method for the Determination of Casein-, Gelatin-, Eggalbumin- and Witte's Peptone-Splitting.

The method used in these cases was essentially the same as LINDERSTRÖM-LANG and SATO's⁽¹⁾⁽³⁾⁽⁴⁾ semi-micro titration method, the principle of which was fundamentally due to the alcohol titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ.⁽⁹⁾ The double digestion vessels⁽¹⁾⁽⁴⁾ which consist of two limbs were also used.

The important points of LINDERSTRÖM-LANG and SATO's method are as follows:

The total volume of the digestion mixture is 5 cc. From this mixture, every 2 cc are pipetted off before and after digestion and poured into 10 cc of 96% alcohol containing 0.4 cc of 0.5% thymolphthalein solution and the increase of carboxyl groups per 2 cc of the digest is measured by the titration with 90% alcoholic $n/20$ KOH solution. The titration was completed after the addition of further more 20 cc of 96% alcohol.

But according to the kind of substrate used the authors have somewhat improved this method as stated in the following, and better reproducible results were obtained.

1. Method for the Determination of Casein-, Eggalbumin- and Witte's Peptone-Splitting.

When the digestion mixture was added into 10 cc of unwarmed 96% alcohol, there occurred suddenly a flocculent separation of the

substrates, the separation of which was not desirable for an accurate titration. Therefore in order to prevent this flocculation, the mixture was added into 10 cc of hot 96% alcohol which was previously warmed to ca. 70° in ca. 10 minutes. The digestion mixture was thus made into homogeneous fine suspensions, which was warmed again to ca. 70° before titration, 20 cc of 96% alcohol which were used for the second addition were not necessary to be warmed to ca. 70°.

2. Method for the Determination of Gelatin Splitting.

When the digestion mixture is added into 10 cc of 96% alcohol, the gelatin coagulates and adheres to the wall of the vessels. This was the case even when 10 cc of 96% alcohol were previously warmed to ca. 70°, WALDSCHMIDT-LEITZ^(*) recommended to add CaCl_2 solution to the mixture in order to prevent coagulation, but this addition seems to us very unfavorable owing to the voluminous precipitation of the mixture thus made and the consequent insensibility of the indicator.

According to our preliminary tests it was found, that the coagulation of gelatin solution is concerned with its pH and alcohol concentration. When the unwarmed alcohol concentration is below 75% and acidic above pH 3.0 the addition of the gelatin solution into alcohol does not cause any coagulation, thus making it possible to keep the mixture for later titration preferably. Therefore, 10 cc of unwarmed 96% alcohol were previously mixed with 1 cc of $n/5$ HCl in the case of using a buffer of pH 3.0-8.0 and 1 cc of $2n/5$ HCl in the case of using a buffer of pH value above 8.0, thus making the pH below 3.0 and alcohol concentration 75% at the stage. 20 cc of boiling 96% alcohol were then added for the completion of the titration. The final alcohol concentration was ca. 88.5%. Other points of the experiment are similar to those of (1).

B. Method for the Determination of Haemoglobin Splitting.

When the haemoglobin was used as substrate, the determination

was followed by means of the Van Slyke's amino nitrogen apparatus, with the micro-burette having a total capacity of 2 cc graduated in 0.01 cc divisions. Total volume of the digestion mixture is 5 cc and every 2 cc are pipetted off before and after digestion and poured into a small tube placed previously in a boiling water-bath and heated for 10 minutes, in order to stop enzyme actions. This whole mixture was shaken for 10 minutes in the apparatus with the addition of 10 drops of normal octyl alcohol (Merck) as foam-stopper.

A half of the increase of the volume of nitrogen gas (corrected to the standard state) due to the digestion, is determined as nitrogen from the NH_2 groups liberated by digestion experiment.

C. pH-regulation of the Digestion Mixture.

1. The Survey of the Buffer Solutions Employed.

The survey of the buffer solutions employed is given in the following table.

TABLE 2.
The survey of the buffer solutions employed.

No.	Buffer solutions	pH-range	pH-determination	Substrate employed
1	Sorensen's citrate buffer (M 5)	6.0	Q. at 20°	Casein
2	" phosphate " (M 3)	=7.0	"	Gelatin
3	$\text{NH}_4\text{Cl-NH}_4\text{OH}$ buffer (n 1)	~7.4	C. at 40°	Eggalbumin Witte's pepton
4	Sorensen's phosphate buffer M 5	4 7.9	Q. at 20°	Haemoglobin
5	" borate** " (M 5)	>8.0	H. at 20°	

Note:—

* Q. pH-determination was made by quinhydrone method.

C.=pH-determination was made by colorimetric method.

H.—pH-determination was made by hydrogen electrode method.

** Addition of glycerine solution must be avoided in the digestion mixture where borate buffer was used, owing to the fact that a borate buffer was effected by glycerine solution.

2. Procedure of pH-regulation of Digestion Mixture.

In order to prepare the digestion mixture of required pH, 2.5 cc of each substrate solution except No. 2 (Table 1, p. 86) were introduced into one limb of the double vessel and mixed with varying amounts of either $n/2$, $n/5$, $n/10$, $n/20$ HCl or $n/5$, $n/10$, $n/20$ NaOH solution required for the pH-regulation of each substrate, as well as for that of venom solutions. The amounts of HCl or NaOH required were found from Figs. 1-7 which corresponded respectively to the Tables 5-11 (pp. 97-99). Then 1 cc of buffer solution was mixed, unless otherwise duly noted, to the above mixture. Into the other limb of the digestion vessel were placed 1 cc of the venom solution and a varying amount of water so as to make the total volume 5 cc.

Fig. 1.

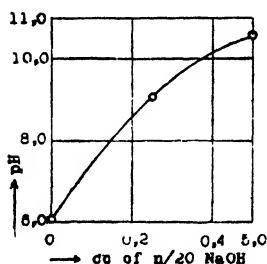


Fig. 2.

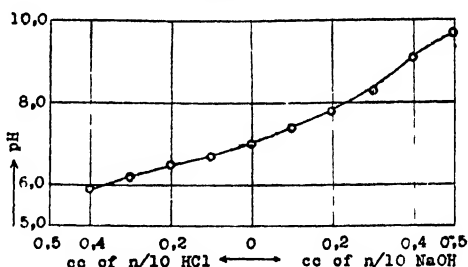


Fig. 3.

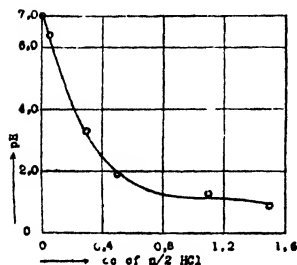


Fig. 4.

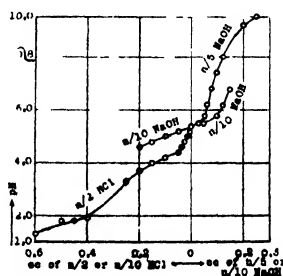


Fig. 5.

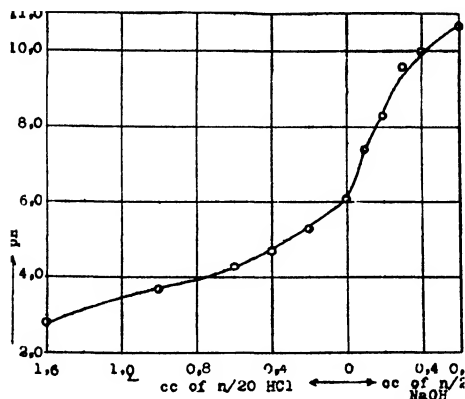


Fig. 6.

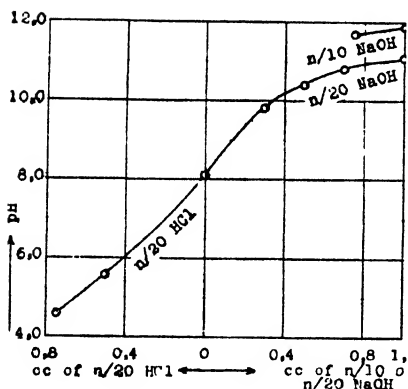


Fig. 7.

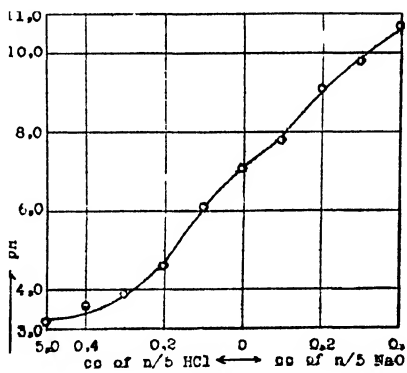


Fig. 1. pH-regulation of 1% venom 30% glycerine solution.

1 cc of the venom solution was mixed with a quantity of n/20 NaOH read from the abscissa and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 5, p. 97)

Fig. 2. pH-regulation of the casein-NaOH solution. (1)

2.5 cc of 6% casein-NaOH solution were mixed with a quantity of n/10 HCl or n/10 NaOH read from the abscissa and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 6, p. 97)

Fig. 3. pH-regulation of the casein-NaOH solution. (2)

2.5 cc of 6% casein-NaOH solution were mixed with a quantity of n/2 HCl read from the abscissa and varying amounts of water to make the

total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 6, p. 97)

Fig. 4. pH-regulation of 6% gelatin solution.

2.5 cc of 6% gelatin solution were mixed with a quantity of $n/2$, $n/10$ HCl or $n/5$, $n/10$ NaOH read from the abscissa and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Tables 7 and 8, pp. 97-98)

Fig. 5. pH-regulation of 3% eggalbumin solution.

2.5 cc of 3% eggalbumin solution were mixed with a quantity of $n/20$ HCl or $n/20$ NaOH read from the abscissa and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 9, p. 98)

Fig. 6. pH-regulation of 3% haemoglobin solution.

2.5 cc of 3% haemoglobin solution was mixed with a quantity of $n/20$ HCl or $n/10$, $n/20$ NaOH and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 10, p. 98)

Fig. 7. pH-regulation of 3% Witte's peptone solution.

2.5 cc of 3% Witte's peptone solution was mixed with a quantity of n 5 HCl or $n/5$ NaOH read from the abscissa and varying amounts of water to make the total volume 5 cc, and the pH value of each mixture was obtained from the ordinate. (cf. Table 11, p. 99)

D. On the Method for the Determination of Stability of Casein Splitting Enzyme of the Snake Venom.

1. *pH-regulation of the Venom for Stability experiment.*

In one limb of a large double vessel were introduced 5 cc of 1% venom (30%) glycerine solution and in the other limb of the vessel were placed 5 cc of water (a) or 1 cc of $n/1$ $\text{NH}_4\text{Cl}-\text{NH}_4\text{OH}$ (8:1) buffer and 4 cc of water (b) or 1 cc of $n/1$ $\text{NH}_4\text{Cl}-\text{NH}_4\text{OH}$ (1:16) and 4 cc of water (c).

The contents of both limbs were mixed and kept standing in a water thermostat at 40° for varying lengths of time. The pH of each mixture was, (a) 6.1, (b) 8.0, and (c) 10.1.

2. *On the Determination of the Stability.*

After varying lengths of time, 1 cc of each mixture was pipetted

off and transferred into one limb of a small double vessel in which 1 cc of 15% glycerine was placed in order to make $CG=6\%$ and 0.5 cc of water, in the case of using the venom regulated as above to pH 6.1 or 8.0 in order to make the pH-value of the digestion mixture 8.0 in the following digestion experiment. In the case of using the venom regulated to pH 10.1, it was necessary to add 0.5 cc of $n/1$ NH_4Cl instead of water for the same purpose. To the other limb of the small double vessel were placed 2.5 cc of 6% casein-pH 8.0 buffer solution (No. 2, Table 1). Then the digestion experiment was carried out as usual for 1 hour at 40° .

E. Symbols.

For the sake of reference, we state here the symbols used in the preceding and following sections.

CG = Glycerine concentration of the digestion mixture.

Cs = Substrate concentration of the digestion mixture.

Cv = mg of the dried venom in 2 cc of the digestion mixture.

X = Number of carboxyl groups formed by the splitting up of the substrate under the given conditions, expressed in cc of $n/20$ KOH per every 2 cc of the digestion mixture.

III. EXPERIMENTAL RESULTS.

A. On the pH-activity-relations of the Proteolytic Enzymes of the Snake Venom.

The relations are clearly illustrated by the pH-activity-curves in Figs. 8 and 9 (p. 95), which correspond to the Tables 12-17 (pp. 99-101). No further comment is necessary except with regard to the following points.

The experiment was carried out for the purpose of mainly finding the optimal pH for the splitting up of each substrate and not to make

strict comparison between the digestibilities of these substrates. Therefore, the conditions of the digestion were made somewhat different from each other according to the substrate used. For the sake of reference, these differences are summarized in the following table.

TABLE 3.

Substrate	C _s %	C _v mg	C _G %	Time of digestion (hrs.)
Casein	3	2	6	1
Gelatin	3	4	6	1
Eggalbumin	1.5	4	6	3
Haemoglobin	1.5	4	0	3
Witte's peptone	1.5	4	6	1

B. On the Relations between the Amount of the Snake Venom or the Time of Digestion and the splitting of Casein.

In the preceding experiment, it was found that casein is most digestible of all the substrates tested. Therefore, in the present experiment the relations named were also examined. These relations are clearly shown in Fig. 10 which corresponds to Tables 18 and 19. (pp. 101-102).

C. On the Stability of the Casein-splitting Enzyme of the Snake Venom.

The stability was also examined when 0.5% venom (15%) glycerine extract of the snake venom was kept standing for several lengths of time (hrs.). The result is given in Fig. 11, which corresponds to Table 20. (p. 102).

Fig. 8.

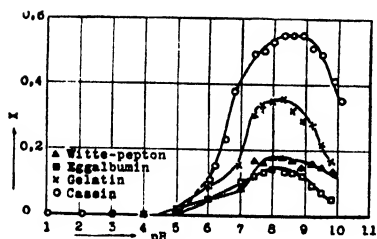


Fig. 9.

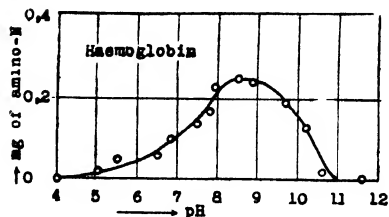


Fig. 10.

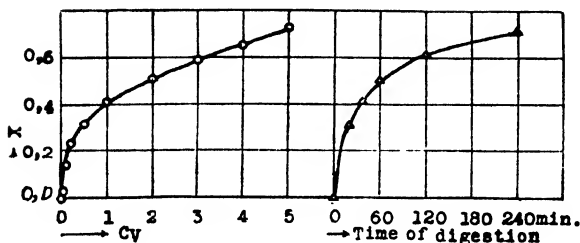


Fig. 11.

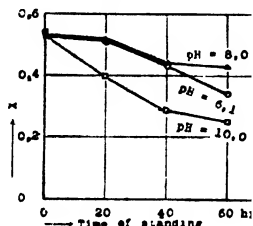


Fig. 8. pH-activity-curves of the proteolytic enzymes of the snake venom. (cf. Tables 12-16, pp. 99-101)

Fig. 9. pH-activity-curves of the proteolytic enzymes of the snake venom. (cf. Tables 17, p. 101)

Fig. 10. Relations between the amount of the snake venom or the time of digestion and the splitting of casein. (cf. Tables 18 and 19, pp. 101-102)

Fig. 11. Stability of the casein-splitting enzyme of the snake venom. (cf. Table 20, p. 102)

SUMMARY.

1. The proteolytic enzymes in the venom of the snake (Taiwan-habu: *Trimeresurus mucrosquamatus*, Cantor) were examined and found positive by the method based on the chemical change of the substrate. As the substrates were used casein, gelatin, eggalbumin, Witte's peptone and haemoglobin, etc.

2. On the chemical method employed. In the case of using haemoglobin as substrate, Van Slyke's method for the micro determination of amino nitrogen was adopted but for the substrates other

than haemoglobin, LINDERSTRÖM-LANG and SATO's⁽¹⁾⁽²⁾⁽³⁾ method was applied after we had made somewhat useful modifications according to the nature of each substrate. These points of modifications were described in detail on pp. 87-88, but some important points of these are summarized below :

In the case of using casein, eggalbumin and Witte's peptone as substrate, 10 cc of 96% alcohol in which the digestion mixture was to be added for the first titration before and after digestion, had previously been warmed to ca. 70° in ca. 10 minutes. 20 cc of 96% alcohol to be added for the completion of the titration were not, as the ordinary method, necessary to be warmed.

When gelatin was used as substrate, it was necessary to use 10 cc of unwarmed 96% alcohol acidified either with 1 cc of $n/5$ HCl or $2n/5$ HCl according to the pH of the buffer used. This was for the purpose of making the pH-value below 3.0 and the alcohol conc. 75% just for the first titration.

For the completion of the titration, 20 cc of boiled 96% alcohol had to be added and immediately titrated.

3. On the optimal pH.

The optimal pH for the splitting of the substrates examined are summarized below, according to the pH-activity-curves in Figs. 8 and 9.

TABLE 4.

Substrate	Optimal pH
Casein	8.3-8.5
Gelatin	8.0-8.3
Eggalbumin	8.0
Haemoglobin	8.5
Witte's peptone	8.0-8.3

4. In the case of casein, the relations between the amount of the snake venom or the time of digestion and the splitting of casein were also examined and shown in Fig. 10.

5. Under the conditions tested, the casein-splitting enzyme of the

snake venom was most stable at pH 8.0 and became unstable as the pH became either more acidic or more alkaline, i. e. pH 6.1 or 10.1. Fig. 11).

TABLE 5.
pH-regulation of 1% venom (30%) glycerine solution.

Venom cc	n'20 NaOH cc	Water cc	pH (H. at 20°)
1	0	4.0	6.1
1	0.25	3.75	9.1
1	0.5	3.5	10.6

TABLE 6.
pH-regulation of the casein-NaOH solution.

2.5 cc of casein-NaOH solution were mixed with a or b or c								
a		pH (H. at 20°)	b		pH H. at 20°	c		pH H. at 20°
n 10 HCl cc	Water cc		n 10 NaOH cc	Water cc		n/2 HCl cc	Water cc	
0	2.5	7.0	0.1	2.4	7.4	0.05	2.45	6.4
0.1	2.4	6.7	0.2	2.3	7.8	0.30	2.20	3.3
0.2	2.3	6.5	0.3	2.2	8.3	0.50	2.00	1.9
0.3	2.2	6.2	0.4	2.1	9.1	1.00	1.50	1.3
0.4	2.1	5.9	0.5	2.0	9.7	1.5	1.00	0.9

TABLE 7.
pH-regulation of gelatin solution. (1)

2.5 cc of gelatin solution were mixed with either a or b					
a		pH (C. at 40°)	b		pH C. at 40°)
n, 10 NaOH cc	Water cc		n 5 NaOH cc	Water cc	
0	2.5	5.4	0.1	2.4	7.4
0.05	2.45	5.5	0.12	2.38	8.0
0.1	2.4	5.8	0.2	2.3	9.2
0.12	2.38	6.2	0.25	2.25	9.5
0.15	2.35	6.8			

TABLE 8.
pH-regulation of gelatin solution. (2)

2.5 cc of gelatin solution were mixed with a or b or c								
a		pH (C. at 40°)	b		pH (C. at 40°)	c		pH (C. at 40°)
n/10 HCl cc	Water cc		n/2 HCl cc	Water cc		n/2 HCl cc	Water cc	
0.05	2.45	5.2	0.05	2.45	4.4	0.4	2.1	1.9
0.1	2.4	5.0	0.1	2.4	4.2	0.45	2.05	1.8
0.15	2.35	4.8	0.15	2.35	4.0	0.5	2.0	1.8
0.2	2.3	4.6	0.2	2.3	3.7	0.6	1.9	1.3
			0.25	2.25	3.3			

TABLE 9.
pH-regulation of 3% eggalbumin solution.

2.5 cc of eggalbumin solution were mixed with either a or b					
a		pH (Q. at 20°)	b		pH (Q. at 20°)
n/20 NaOH cc	Water cc		n/20 HCl cc	Water cc	
0	2.5	6.1	0.2	2.3	5.3
0.1	2.4	7.4	0.4	2.1	4.7
0.2	2.3	8.3	0.6	1.9	4.3
0.3	2.2	9.6	1.0	1.5	3.7
0.4	2.1	10.0	1.6	0.9	2.8
0.6	1.9	10.7			

TABLE 10.
pH-regulation of 3% haemoglobin solution

2.5 cc of 3% haemoglobin solution were mixed with a or b or c								
a		pH (H. at 20°)	b		pH (H. at 20°)	c		pH (H. at 20°)
n/20 NaOH cc	Water cc		n/10 NaOH cc	Water cc		n/20 HCl cc	Water cc	
1.0	1.5	11.1	1.0	1.5	11.9	0.5	2.0	5.6
0.7	1.8	10.8	0.75	1.75	11.7	0.75	1.75	4.6
0.5	2.0	10.4						
0.3	2.2	9.8						
0	2.5	8.1						

TABLE 11.
pH-regulation of 3% Witte's peptone solution.

2.5 cc of Witte's peptone were mixed with either a or b					
a		pH (H. at 20°)	b		pH (H. at 20°)
n/5 NaOH cc	Water cc		n/5 HCl cc	Water cc	
0	2.5	7.1	0.1	2.4	6.1
0.1	2.4	7.8	0.2	2.3	4.6
0.2	2.3	9.1	0.3	2.2	3.9
0.3	2.2	9.8	0.4	2.1	3.6
0.4	2.1	10.7	0.5	2.0	3.2

Note on Tables 5-11:

C.=pH-determination was made by the colorimetric method.

H. pH-determination was made by the hydrogen electrode method.

Q. pH-determination was made by the quinhydrone method.

On the further details of pH-determination, cf.⁽⁴⁾ especially p. 23.

TABLE 12.
Casein splitting (1).

C_s -3%, C_v -2.0 mg, C_r =6%.

pH of the digestion mixture was regulated with n/2 or n/10 HCl without any buffer solutions, and determined by the hydrogen electrode method. Digestion for 1 hour at 40°.

pH before and after digestion		X	pH before and after digestion		X
1.0	1.0	0	6.0	6.0	0.11
2.1	2.1	0	6.3	6.1	0.15
3.0	3.0	0	6.6	6.5	0.23
			6.9	6.7	0.38

Digestion experiment was not carried out at the pH-range between 3-6, because the casein solution coagulated flocculently at this pH range.

TABLE 13.
Casein splitting (2).

$C_s = 3\%$, $C_v = 2.0$ mg, $C_G = 6\%$.

Buffer solution No. 3 (Table 2) was used. Digestion for 1 hour at 40° .

pH before and after digestion		X	pH before and after digestion		X
7.4	7.4	0.49	8.9	8.9	0.55
7.7	7.7	0.50	9.2	9.2	0.51
8.0	8.0	0.53	9.5	9.5	0.49
8.3	8.3	0.55	9.8	9.8	0.41
8.6	8.6	0.55	10.1	10.1	0.35

TABLE 14.
Gelatin splitting.

$C_s = 3\%$, $C_v = 4.0$ mg., $C_G = 6\%$.

Buffer solutions No. 1-3 (Table 2) were used. Digestion for 1 hour at 40° .

pH before and after digestion		X	pH before and after digestion		X
3.0	3.0	0	8.0	8.0	0.35
4.0	4.0	0	8.3	8.3	0.36
5.0	5.0	0.02	8.6	8.6	0.32
6.0	6.0	0.09	8.9	8.9	0.29
7.0	7.0	0.15	9.2	9.2	0.28
7.4	7.4	0.31	9.5	9.5	0.22
7.7	7.7	0.34	9.8	9.8	0.17

TABLE 15.
Eggalbumin splitting.

$C_s = 1.5\%$, $C_v = 4.0$ mg., $C_G = 6\%$.

Buffer solutions No. 1-3 (Table 2) were used. Digestion for 3 hours at 40° .

pH before and after digestion		X	pH before and after digestion		X
3.0	3.0	0	8.0	8.0	0.14
4.0	4.0	0	8.3	8.3	0.13
5.0	5.0	0	8.6	8.6	0.13
6.0	6.0	0.05	8.9	8.9	0.12
7.0	7.0	0.10	9.2	9.2	0.09
7.4	7.4	0.12	9.5	9.5	0.07
7.7	7.7	0.13	9.8	9.8	0.05

TABLE 16.

Witte's peptone splitting.

C_s =1.5%, C_v =4.0 mg., C_G =6%.

Buffer solutions No. 1-3 (Table 2) were used. Digestion for 1 hour at 40°.

pH before and after digestion			X	pH before and after digestion			X
3.0	3.0		0	8.0	8.0		0.23
4.0	4.0		0	8.3	8.3		0.18
5.0	5.0		0.02	8.6	8.6		0.17
6.0	6.0		0.05	8.9	8.9		0.15
7.0	7.0		0.08	9.2	9.2		0.16
7.4	7.4		0.17	9.5	9.5		0.15
7.7	7.7		0.16	9.8	9.8		0.14

TABLE 17.

Haemoglobin splitting.

C_s =1.5%, C_v = 4.0 mg., C_G =0.

Buffer solution 4-5 (Table 2) were used. Digestion for 3 hours at 40°.

pH before and after digestion			Increase of amino N in mg	pH before and after digestion			Increase of amino N in mg
4.0	4.0		0	7.9	8.0		0.23
5.0	5.0		0.02	8.5	8.5		0.25
5.5	5.5		0.05	8.9	8.9		0.24
6.5	6.5		0.06	9.7	9.7		0.19
6.8	6.8		0.10	10.2	10.2		0.13
7.7	7.3		0.14	10.6	10.6		0.02
7.9	7.8		0.17	11.6	11.6		0

TABLE 18.

Relation between the amount of snake venom and the splitting of casein. C_G=15%, C_S=3%, pH=8.0. 6% casein-pH 8.0 buffer solution No. 2 (Table 1) was used. Varying amount of 1% venom (30%) glycerine solution were used. Digestion for 1 hour at 40°.

Cv	X	Cv	X
5.0	0.73	0.5	0.32
4.0	0.66	0.2	0.23
3.0	0.59	0.1	0.14
2.0	0.51	0.02	0.02
1.0	0.41	0	0

TABLE 19.

Relation between the time of digestion and the splitting of casein. CG=6%, CV=2.0 mg., pH=8.0. 6% casein-pH 8.0 buffer solution No. 2 (Table 1) was used for Xa or Xb, and 1 cc of buffer solution No. 3 (Table 2) for Xc.

Time of digestion in min. at 40°	Xa	Xb	Xc	X-Xa-Xb-Xc
	Casein, Buffer Venom	Buffer Casein	Buffer Venom	
0	0	0	0	0
18	0.31	—	—	0.31
36	0.41	—	—	0.41
60	0.52	0.01	—	0.51
120	0.66	0.03	0.01	0.62
240	0.85	0.09	0.06	0.70

Xa, Xb and Xc are the values corresponding to X (cf. Symbols) at each different mixture in each column.

TABLE 20.

The stability of the casein-splitting enzyme of the snake venom.

For stability: 0.5% venom (15%) glycerine solution was kept standing at 40° for varying lengths of time.

For digestion experiment: CG=6%, CV=2.0 mg., CS=3%, pH=8.0. 6% casein-pH 8.0 buffer solution No. 2 (Table 1) was used. Digestion for 1 hour at 40°.

Time of standing in hours	pH of venom solution	X
0	6.1	0.53
	8.0	0.53
	10.1	0.54
20	6.1	0.51
	8.0	0.52
	10.1	0.40
40	6.1	0.43
	8.0	0.44
	10.1	0.29
60	6.1	0.34
	8.0	0.43
	10.1	0.25

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ENZYME CHEMICAL INVESTIGATION OF FORMOSAN SNAKE VENOMS.

II. On the Activation of Trypsin by the Snake Venoms.

(With 12 Text-figures)

Masakazu SATO and Tamotu HIRANO

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INTRODUCTION

We considered that investigations on the specific effect (activation or inhibition) of the snake venoms upon the activity of enzymes must be one of the most interesting lines of great significance, of the enzyme chemical investigations of the snake venoms. Starting from this consideration, we searched for the literature and found that there were only two investigations as stated below. DELEZENNE⁽¹⁾ (1902) has shown that snake venom contains a kinase which activates the inactive pancreatic juice, enabling the latter energetically to digest albumin. According to his investigation, 1-5 mg of lachesis venom added to 1 cc of inactive pancreatic trypsin could cause a complete digestion of 0.5 g albumin within 10-12 hours, and even 0.0125 mg of lachesis venom added could make the same effect in 72 hours, while cobra venom was less active and pelias venom required about 5 times as much to obtain the same effect. LAUNOY⁽²⁾ (1902) found that cobra venom has no activation power upon soluble ferments such as emulsin, amylase and pancreatin.

According to these records, the activation power of the venoms was mainly found upon the pancreatic trypsin, but these investigations were made however at the time when the rational and accurate method for the determination of the proteolytic enzyme was not yet completely established. And thus, the determination was done by a method based rather on the physical change and not on the chemical change of the substrate.

Besides, no fundamental research had yet been done, even on the activation effect itself. Therefore, we undertook the present investigation, using, on the one hand, Formosan snake venoms which were collected in our laboratory, and using, on the other hand, glycerine extract of the pig's pancreas as trypsin solution. For the determination of the proteolytic activity was used of course rational and accurate method which we established and described in our previous paper,⁽³⁾ and the activation effect was tested also in detail from the view point of modern enzyme chemistry. Consequently, we obtained many interesting results which are of great significance. These are summarized on p. 125.

It is our pleasant duty to express our cordial thanks to the Imperial Academy, for the subsidy we received for our researches.

EXPERIMENTAL PART

I. METHODS OF PREPARATION

A. Preparation of the Snake Venom Solution.

1. Preparation of the Dried Powder of Venoms.

In the main part of this experiment, unless otherwise duly noted, the venom of the snake Taiwan-habu (*Trimeresurus mucrosuquamatus*, CANTOR) was used, though we used for comparison, in the latter part of this experiment, the venoms of various kinds of snakes such as Hyappo-da (*Agkistrodon actus*, GRÜNTHER), Ao-hebi (*Trimeresurus graminens*, SHAW), Taiwan-kobura (*Naja naja atra*, CANTOR), and Amagasa-hebi (*Bungarus multicinctus*, BLYTH) etc. The latter two

belong to the Colubridae and the others to the Viperidae. Each fresh venom was a viscous liquid. We dried it in a vacuum desiccator over CaCl_2 under the reduced pressure. Each dried venom powder was of a white to yellow colour, according to the kind of snake.

2. Preparation of the Snake Venom Solution.

0.1% venom water solution was prepared and after being thoroughly stirred, centrifuged and filtered off. The clear solution thus obtained was kept for use in a refrigerating chamber at 0° .

B. Preparation of Trypsin Solution.

1. Preparation of the Dried Powder of Pig's Pancreas.

Fresh pancreas of pig which was obtained from the Dairyûdo-slaughter house at Taihoku, was passed repeatedly through a meat-mincer after being separated from the contaminated fat and muscle, and ground in a porcelain mortar into porridge-like condition. The porridge thus obtained was dried with acetone and ether according to WILLSTÄTTER and WALDSCHMIDT-LEITZ.⁽⁶⁾ For instance, 1 kg of the ground pancreas porridge was treated twice with 2 L of acetone, then twice with a mixture of 1 L of acetone and 1 L of ether and finally twice with 2 L of ether. Each operation was performed as rapidly as possible and the suspension was filtered immediately without allowing it to stand. The product thus obtained was dried between filter papers and ground into fine powder which was passed through a sieve of 1 mm mesh and preserved for use in a desiccator. The water content of the dried powder amounted to 11.67 %.

2. Preparation of Trypsin Solution.

2 g of the pancreas powder above obtained were suspended in 100 cc of glycerine and ground thoroughly in a porcelain mortar. The mixture was centrifuged and filtered off, after allowing it to stand (for ca. 10 minutes) at room temperature. The filtrate was preserved as the stock solution, with the addition of 1 % toluene in a refrigerating chamber at 0° . Just before the digestion experiment,

this 60 % glycerine extract was diluted with an equal volume of water for the preparation of 1 % pancreas (30 %) glycerine solution. For the preparation of pancreas solutions, the concentration of which being lower than 1 %, this stock solution was further diluted with 30 % glycerine solution.

3. *Preparation of Trypsin Solution from Commercial Trypsin.*

As commercial trypsin, were employed two preparations, i. e. E. Merck's (Darmstadt) as well as Dr. G. Grübler & Co.'s (Leipzig). Trypsin solutions were prepared from these substances in quite a similar way to the previous section.

2.5 g of the commercial trypsin were suspended in 100 cc of 30 % glycerine and ground thoroughly in a porcelain mortar. The mixture was centrifuged and filtered off, after allowing it to stand (for ca. 10 minutes) at room temperature. The filtrate was preserved with the addition of 1 % toluene in a refrigerating chamber at 0°, and used without any dilution.

C. Preparation of Substrate solutions.

Preparation of substrate solutions was carried out in exactly the same way as that previously described.⁽³⁾

Substrate solutions prepared were given in the following table.

TABLE 1.
Survey of the substrate solutions prepared.

No.	Substrate solutions	pH
1.	6% casein solution	7.0 at 20°
2.	6 % casein-pH 8.0 buffer solution.	8.0 (at 40°
3.	6 % gelatin solution.	5.4 at 20°
4.	3 % Witte's peptone solution.	7.4 at 20°

II. ANALYTICAL METHODS

On the determination of the proteolytic enzyme activity. For this purpose, was used the same method as that employed in a pre-

vious paper.⁽¹⁾ The only difference from that is to add, besides the digestion experiment, the process of activation of the enzyme, in which the venom solution and the enzyme solution were made in contact for a definite length of time.

Casein was used as substrate in the main part of this experiment but gelatin or Witte's peptone was also used sometimes.

In the following, only important points are to be noted. The total volume of the digestion mixture is 5 cc. From this mixture, every 2 cc are pipetted off before and after digestion and the increase of carboxyl groups per 2cc of the digest is measured by the titration with 90 % alcoholic n/20 KOH.

The digestion was carried out for 1 hour at 40'. Before that, the activation was made also for 1 hour at 40', unless otherwise is duly noted.

III. SYMBOLS

For the sake of reference, we state here the symbols used in the preceding and following sections.

C_E = Enzyme concentration reduced to mg of dried pancreas powder used to prepare that amount of enzyme extract per every 2 cc of digestion mixture.

C_E' = enzyme concentration reduced to mg of dried pancreas powder used to prepare that amount of enzyme extract per every 2 cc of activation mixture.

$$C_E' = 2 C_E$$

C_G = glycerine concentration of the mixture of digestion or activation.

C_S = substrate concentration of the digestion mixture.

C_V = mg of the dried venom in 2 cc of the digestion mixture.

C_V' = mg of the dried venom in 2 cc of the activation mixture.

$$C_V' = 2 C_V$$

X = number of carboxyl groups formed by the splitting of the substrate under the given conditions, expressed in cc of n/20 KOH per every 2 cc of the digestion mixture.

IV. EXPERIMENTAL PROCESSES AND RESULTS.

A. On the Time Required for Full Activation of Trypsin by the Snake Venom.

1. *Test of the Activation made for Comparatively Long Time.*

Process.

In this case, the venom of Taiwan-habu and the trypsin solution of the dried powder of pig's pancreas were used.

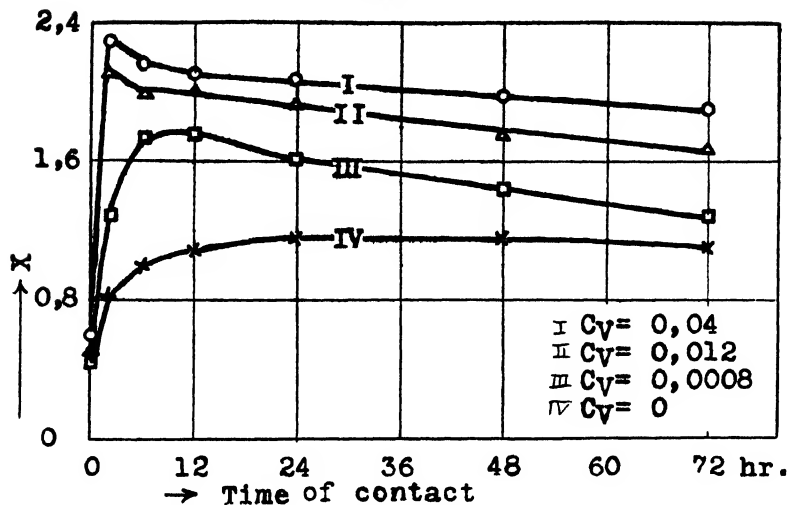
In one limb of each large digestion vessel were introduced 1.5×8 cc of 1 % pancreas (30 %) glycerine solution and in the other limb of the vessel was taken 1×8 cc of the venom water solution of various concentration, and after mixing the contents in both limbs the mixture was kept standing at 40 for varying lengths of time such as from 0 to 72 hours. Just ca. 10 minutes before the digestion experiment at each interval, 2.5 cc of pancreas-venom solution treated as above were put in one limb of each small digestion vessel, and 2.5 cc of 6 % casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) were introduced in the other limb of each vessel and brought back immediately into the water thermostat at 40°.

After each interval such as noted in Table 3 (p. 127) the contents of both limbs were mixed thoroughly and the digestion experiment was carried out for 1 hour at 40. In the case of contact, time being 0, 1.5 cc of 1 % pancreas (30 %) glycerine solution were taken in one limb of a double digestion vessel and 1.0 cc of venom water solution of varying concentration and 2.5 cc of 6 % casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) were taken in the other limb, and digestion experiment was carried out in the same way as above.

Experimental results.

The result is given in Table 3 (p. 127) and is illustrated in Fig. 1 (p. 112). According to the curves in Fig. 1, the full activation is made within 2 hours. The curve where $C_v=0$, corresponds to the spontaneous activation of the trypsin preparation itself, the spontaneous activation of which is however not so marked as that of trypsin by the venom.

Fig. 1.



Time required for full activation of trypsin by the snake venom. Test of the activation made for comparatively long time. (cf. Table 3, p. 127)

2. Test of the Activation made for Comparatively Short Time.

Process.

Since we knew from the result of the previous test that the full activation of the pancreas trypsin by the snake venom was made within 2 hours, we repeated a similar experiment, in order to test the activation made at comparatively short intervals within 4 hours. In a similar way to the previous test, the test was made in each case of using varying concentrations of the snake venom under two different concentrations of the pancreas trypsin.

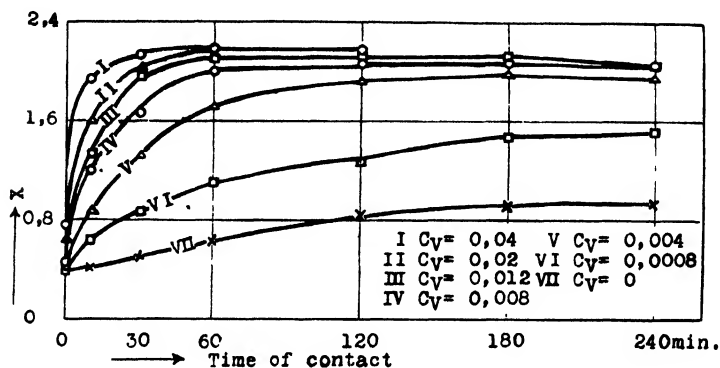
Experimental result.

The result is given in Table 4 (p. 127) and Figs. 2 and 3 (p. 113). According to the curves in Figs. 2 and 3, a pretty remarkable activation is made even when the contact of trypsin with the venom is made for so short an interval as 10 minutes, and the time required for full activation of trypsin is 30-180 minutes at 40° according to C_v and C_E employed. For convenience sake, in the following experiment to find other conditions which have an influence upon the

activation, 60 minutes were taken as the activation time before each digestion experiment.

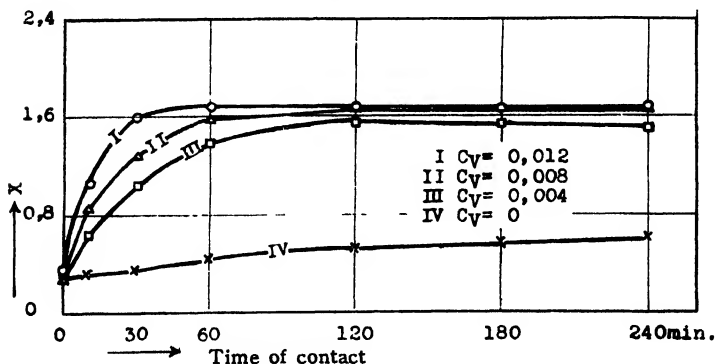
The need of a certain time for activation of trypsin by the venom is in quite similar relationship to that by the enterokinase from the duodenum, the activation of the latter was fully investigated by WALDSCHMIDT-LEITZ,⁽⁴⁾ etc.

Fig. 2.



Time required for full activation of trypsin by the snake venom. Test of the activation made for comparatively short time. (1) (cf. Table 4, p. 127)

Fig. 3.



Time required for full activation of trypsin by the snake venom. Test of the activation made for comparatively short time. (2) (cf. Table 4, p. 127)

B. On the Relation between the Amount of Venom and that of Trypsin for the Full Activation of the Latter.

Process.

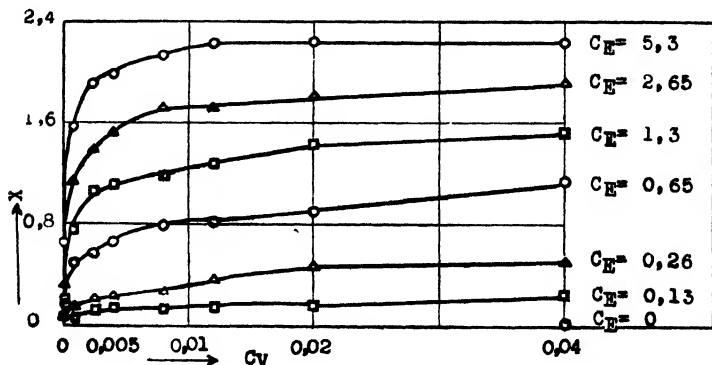
For the purpose of this experiment, water extract of the venom of Taiwan-habu and glycerine extract of the dried powder of pig's pancreas were used. In one limb of each double digestion vessel were placed 2.5 cc of casein-pH 8.0 buffer solution (Table 1, No. 2. p. 109) and in the other limb, were taken 1.5 cc of pancreas glycerine (30 %) solution, the concentration of pancreas being varied from 0 to 1 % corresponding to C_E from 0 to 5.3 mg and was mixed with 1 cc of venom water solution, the concentration of which being varied from 0 to 0.01 % corresponding to C_V from 0 to 0.04 mg.

Each digestion vessel was then kept in a water thermostat at 40'. The activation of trypsin by the venom was made at 40' for 1 hour measured from the time of mixing the trypsin and the venom solutions. Then, the contents of both limbs of each digestion vessel were mixed thoroughly and the digestion experiment was carried out in precisely the same way as usual.

Experimental result.

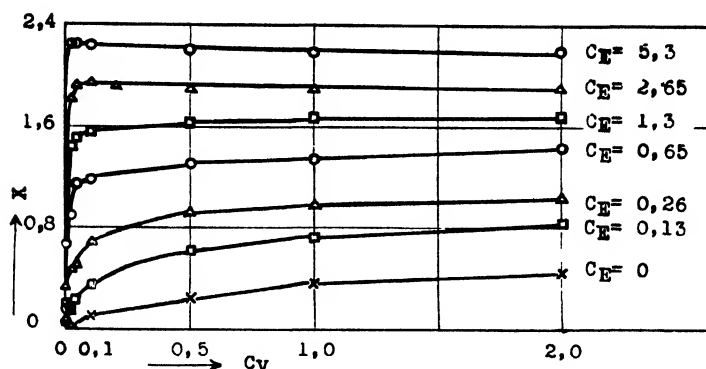
The result is given on Table 5 (p. 128) and is illustrated in Figs. 4 and 5 (pp. 114 and 115). Before judging the data, it should be noticed

Fig. 4.



On the relation between the amount of venom and that of trypsin for the full activation of the latter. (1) (cf. Table 5, p. 128)

Fig. 5.



On the relation between the amount of venom and that of trypsin for the full activation of the latter. 2 (cf. Table 5, p. 128)

here that the venom has not shown any perceptible proteolytic activity in its concentrations lower than C_v 0.04 mg, though it is becoming apparent in its concentration higher than C_v 0.1 mg. It is also noticed here that the proteolytic activity of the venom which appeared in its higher concentrations such as C_v from 0.1 mg to 2.0 mg, has no perceptible influence upon the full activity curves of trypsin, at least in higher concentrations such as C_v 2.65 mg or 5.3 mg.

Judgement of the data.

According to WALDSCHMIDT-LEITZ,⁽¹⁾ in the activation of trypsin by the enterokinase, there existed a definite proportionality between the amount of trypsin and that of enterokinase. According to the data which we obtained, in the activation of trypsin by the venom, such a proportionality was not found at all. This, according to our opinion, may be due to the fact that the venom solution was not yet the so-called "einheitlich" from the standpoint of the kind of its activities. Anyhow, it should be emphasised here that the venom in such a low concentration as C_v 0.04 mg had the sufficient power to make the full activation of trypsin in its concentration such as $C_E = 5.3$ mg.

C. pH-Stability-Relation of the Venom from the Standpoint of its Activation Power upon the Proteolytic Activity of Trypsin.

Process of allowing the venom solutions to stand at varying pH.

For this purpose, the venom of Taiwan-habu was used. In one limb of each large double digestion vessel were introduced 10 cc of 0.04 % venom water solution and in the other limb of each vessel were placed 10 cc of $n/50$ HCl or 10 cc of a mixture consisting of 1 cc of $n/1$ NH_4Cl - NH_4OH buffer of pH 10 and 9 cc of water, in order to regulate the pH of each venom solution to 2 or 10 after the contents of both limbs of each digestion vessel had been mixed.

In a similar way, in one limb of each large double digestion vessel, were taken 10 cc of 0.02 % venom water solution and in the other limb of each vessel were put 10 cc of water or 10 cc of a mixture consisting of 1 cc of $n/1$ NH_4Cl - NH_4OH buffer of pH 8.0 and 9 cc of water, in order to regulate the pH of each venom solution to 6.1 or 8.0 after the contents of both limbs of each digestion vessel had been mixed.

Each mixture, i. e. each venom water solution regulated to varying pH was kept standing in a water thermostat at 40° for varying lengths of time, such as from 0 to 96 hours and then the following processes of activation and digestion were carried out.

Process of activation and digestion.

In one limb of each digestion vessel were introduced 2.5 cc of casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) and in the other limb of each vessel were placed 1.5 cc of 1 % pancreas glycerine (30 %) solution, and was added 1 cc of 0.01 % venom water solution if taken from the venom solution regulated to pH 6.1 or 8.0. In a similar way, if taken from the venom solution regulated to pH 2.0, 0.5 cc of 0.02 % venom solution was added and neutralized with 0.5 cc of $n/100$ NaOH; if taken from the venom solution regulated to pH 10.1, 0.5 cc of 0.02 % venom solution was added and mixed with 0.18 cc of $n/1$ NH_4Cl solution and 0.32 cc of water. These processes

were done for the purpose of regulating the pH of each digestion mixture to 8.0.

Before mixing the contents of each digestion vessel, the activation of trypsin with the venom solution was done for 1 hour at 40° and then digestion was carried out for 1 hour at 40° and the determination was made in precisely the same way as usual.

Process of control experiment.

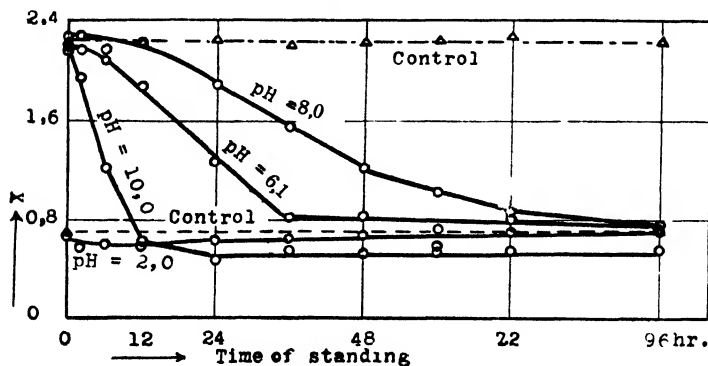
0.01 % venom water solution, the pH of which being 6.1, as well as 1 % pancreas glycerine (30 %) extract, the pH of which being 6.3, were preserved at 0° before use.

Control test was made in a similar way to the previous experiment. pH for activation and that for digestion were 6.2 and 8.0 respectively.

Experimental results.

The result is given on Table 6 (p. 129) and is illustrated in Fig. 6 (p. 117). According to the figure, the venom solution when kept standing at 40° at various pH for varying lengths of time, the activation power upon the proteolytic power of trypsin diminishes almost completely within 96 hours when pH=8.0, within 36 hours when pH 6.1, within 12 hours at pH 10.1, and almost immediately at pH 2.0.

Fig. 6.



pH-stability-relation of the venom. (cf. Table 6, p. 129)

Thus the relation of the stability of the venom at varying pH is shown as follows: $\text{pH } 2.0 < \text{pH } 6.1 < \text{pH } 8.0 > \text{pH } 10.1$.

Note:

In the activation process, pH of each mixture (enzyme and venom) was somewhat varying, such as from 6.1 to 8.0, though the pH of each digestion mixture (enzyme, venom and substrate) was all regulated to 8.0. Therefore test was made on this point, but we could not observe any perceptible influence of such a pH-variation upon the results of the experiment.

D. Thermal Resistance of the Venom Water Solution from the Standpoint of its Activation Power upon the Proteolytic Activity of Trypsin.

Process of testing thermal resistance.

In each test tube were introduced a required amount (ca. 10 cc in this case) of 0.01 % venom water solution (pH 6.1) and kept standing in each water thermostat regulated to various temperature, in a range such as from 40° to 80° for varying lengths of time, such as from 0 to 120 minutes. At each interval, ca. 2 cc of each venom solution were pipetted off from each test tube and poured into each small test tube which was cooled with ice in a beaker and preserved before use for ca. 120 minutes in a refrigerating chamber at 0°.

Process of activation and digestion.

In one limb of each digestion vessel were introduced 2.5 cc of casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) and in the other limb were placed 1.5 cc of 1 % pancreas glycerine (30 %) extract and 1 cc of water in the case of control experiment or 1 cc of 0.01 % venom water solution subjected to thermal resistance in the previous process. Each vessel was kept standing at 40°, 1 hour without mixing and for 1 hour after mixing the contents of both limbs. The former was for the purpose of activation and the latter for the purpose of digestion.

Experimental results.

The result is given on table 7 (p. 130) and is illustrated in Fig. 7 (p. 119). According to Fig. 7 the thermal resistance of the venom water solution tested from the standpoint of its activation power upon the proteolytic activity of trypsin is as follows :

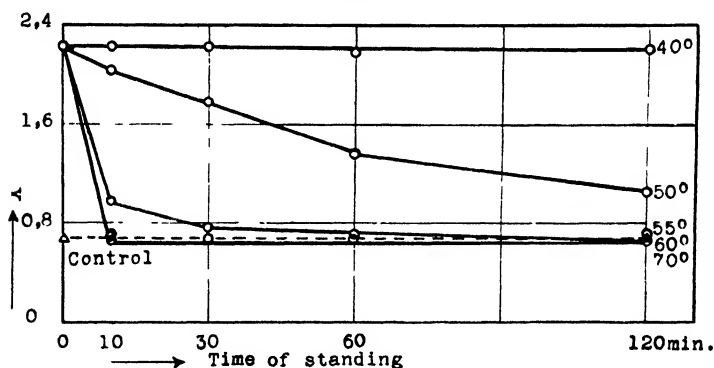
At 40°, the venom solution was most resistant, essentially no change was observed at all within 120 minutes.

At 50°, the resistance of the venom solution was decreasing very slowly.

At 55°, the resistance of the venom solution was decreasing quickly, such as within 30 minutes.

At 56°-80°, the resistance of the venom solution was decreasing very quickly such as within 10 minutes.

Fig. 7.



Thermal resistance of the venom. (cf. Table 7, p. 130)

E. On the Activation Power of the Venom upon the Protelytic Activity of Trypsin of Pig's Pancreas, which was Subjected to its Spontaneous Auto-Activation for Varying Lengths of Time.

Process of auto-activation.

Ca. 20 cc of 1 % pancreas glycerine (30 %) solution were introduced in a vessel and kept standing in a water thermostat at 40° for varying lengths of time.

Process of activation and digestion.

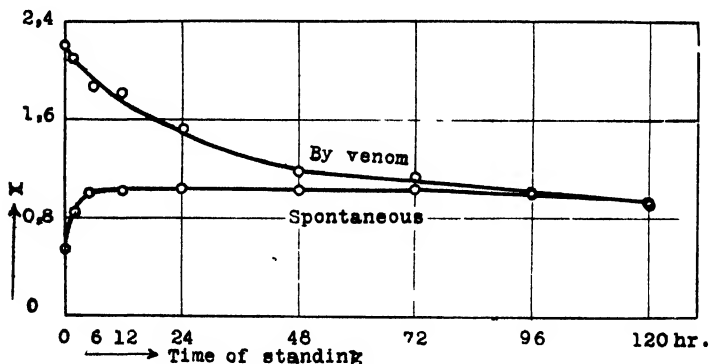
In one limb of each double digestion vessel were placed 2.5 cc of casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) and in the other limb was introduced 1 cc of 0.01 % venom water solution or 1 cc of water and were added 1.5 cc of trypsin solution which were subjected, as above stated, to its auto-activation for varying lengths of time. Further processes of activation and digestion were carried out in precisely the same way as usual, the time being respectively for 1 hour at 40°.

Experimental result.

The result is given on Table 8 (p. 130) and is shown in Fig. 8 (p. 120). As clearly shown from the figure, spontaneous auto-activation of trypsin attained its full equilibrium after the extract had been kept standing at 40° for 6 hours.

Activation power of the venom upon the trypsin was decreasing as the time of the auto-activation of trypsin was lengthening and became practically zero when the time of the auto-activation reached 96 hours.

Fig. 8.



Activation power of the venom upon the proteolytic activity of trypsin of pig's pancreas which was subjected to its spontaneous auto-activation (cf. Table 8, p. 130)

F. Test on the Activation Power of the Venom upon the Proteolytic Activity of Commercial Trypsin.

Process.

Two preparations of commercial trypsin were employed for this purpose. (p. 131). Test was made in exactly the same way as that of the previous section, without subjecting the commercial trypsin to its auto-activation. 1.5 cc of 2.5 % commercial trypsin (30 %) glycerine extract were here used instead of 1 % dried pig's pancreas powder (30 %) glycerine extract in the previous case (E).

Experimental result.

The result is given on Table 9 (p. 131). According to the result, no activation power of the venom was observed upon the proteolytic activity of the commercial trypsin preparations. This, according to our opinion, may be due to the fact that the commercial trypsin preparations were fully activated, which might have happened during the course of preparation. (cf. section E)

G. On the Activation Power of the Venom upon the Proteolytic Activity of Trypsin at Varying pH.

Process.

As the substrate solutions, were used 6 % casein solution, 6 % gelatin solution and 3 % Witte's peptone. (Table 1, No. 1, 3, and 4, p. 109). In one limb of each digestion vessel were placed 0.7 cc of 0.5 % trypsin glycerine (30 %) solution and 0.3 cc of 0.01 % venom water solution. In the other limb of each vessel were introduced 2.5 cc of substrate solution, 1 cc of buffer solution such as noted on Table 2 and 0.5 cc of a mixture of water and $n/5$ NaOH or $n/5$ HCl required in order to bring the pH of the substrate solution to a varying value. The amount (cc) of acid or alkali required for the pH-regulation of the substrates were easily found from the curves given in a previous report.⁽⁷⁾ In the case of using casein as substrate, pH-regulation between 6-7 was done with addition of only $n/10$ HCl without the addition of any buffer, and on the more acidic side than pH 6.0,

digestion experiment was not carried out owing to the fact that precipitation of casein occurred.

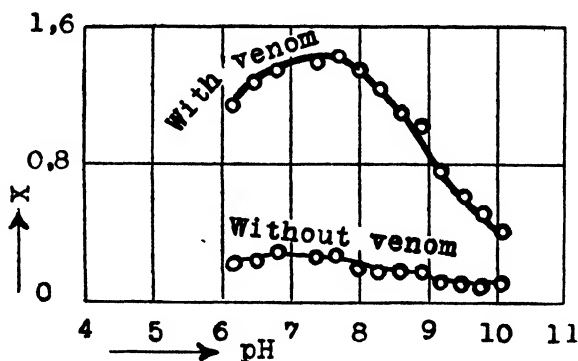
TABLE 2.
Buffer solution used.

pH range	Buffer solutions	Measurement of pH
pH<6	Sorensen's citrate buffer (M/5)	Quinhydrionic, at 25°
pH=7.0	" phosphate " (M/3)	" "
pH>7.4	NH ₄ Cl-NH ₄ OH buffer (n/1)	Colorimetric, at 40°

Experimental result.

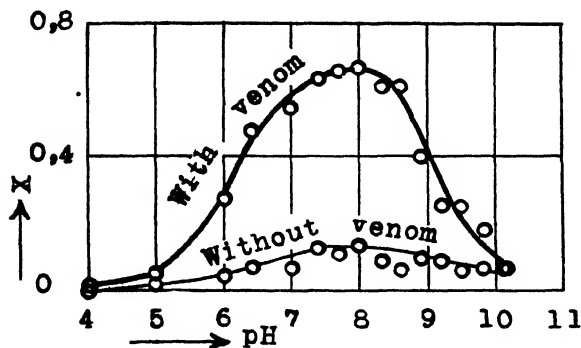
The results are given on Table 10, 11 and 12 (pp. 132 and 133) and in Figs. 9, 10 and 11 (pp. 122 and 123). Generally observing these figures, the optimal pH of trypsin lies between 7-8.0 no matter whether the venom was added as activator or not, and no matter whether any of these substrates was used. And the activation of the venom upon trypsin is most favourable at the pH zone optimal for the activity of trypsin.

Fig. 9.



Activation power of the venom upon the casein splitting activity of trypsin at varying pH. (cf. Table 10, p. 132)

Fig. 10.



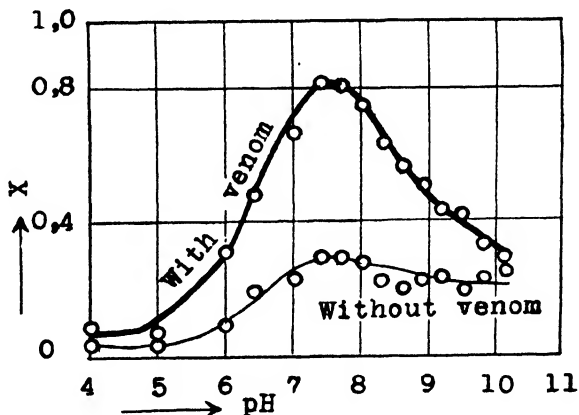
Activation power of the venom upon the gelatin splitting activity of trypsin at varying pH.

(cf. Table 11, p. 132)

Fig. 11.

Activation power of the venom upon the Witte's peptone splitting activity of trypsin at varying pH.

(cf. Table 12, p. 133)



H. Comparison of the Venom of Various Kinds of Snake, From the Standpoint of their Activation Power upon the Proteolytic Activity of Trypsin.

Process.

In this experiment, were employed the venoms of Taiwan-habu Hyappo-da and Ao-hebi (Viperidae) which belong to the so-called "Haemorrhagic venom," as well as the venoms of Taiwan-kobura and Amagasa-hebi (Colubridae) which belong to the so-called "Neurotoxin." In one limb of each double digestion vessel were introduced

2.5 cc of casein-pH 8.0 buffer solution (Table 1, No. 2, p. 109) and in the other limb of each vessel were placed 1.5 cc of 1 % trypsin glycerine (30 %) solution and added 1.0 cc of 0.25 % or 0.01 % venom water solution prepared from each venom of various kinds of snakes.

The process of the activation of trypsin with each venom as well as that of digestion experiment were carried out respectively for 1 hour at 40° precisely in the usual way.

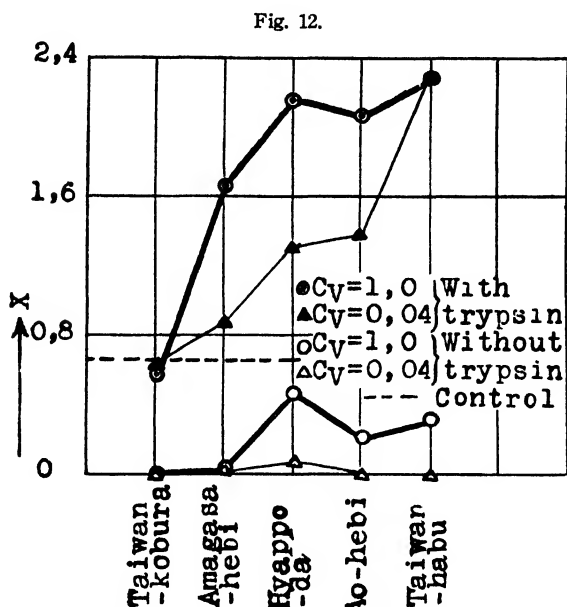
Control experiment was also made without 1.5 cc of trypsin solution in the above digestion mixture but with the addition of 1.5 cc of 30 % glycerine instead. This was for the purpose of determining the proteolytic activity of each venom itself besides its activation power.

Experimental result.

The result is given on Table 13 (p. 134) and is shown in Fig. 12 (p. 125). As clearly shown from Fig. 12, the venoms of most kinds of the snakes i. e. Taiwan-habu, Hyappoda, Ao-hebi and Amagasa-hebi have a remarkable activation power upon the proteolytic activity of trypsin, though it was found that the venom of Taiwan-kobura has absolutely no activation power even in such a high concentration as $C_v=1.0$ mg. This exception is very interesting and attractive when we consider that the venom of Taiwan-kobura belongs to "Neuro-toxin." But care must be taken of the fact that the venom of Amagasa-hebi, which also belongs to "Neuro-toxin" has a pretty high activation power in its higher concentration, such as $C_v=1.0$ mg, though not so marked in its lower concentration, such as $C_v=0.04$ mg.

As to the proteolytic activity of the venom itself, the venoms of Taiwan-kobura as well as that of Amagasa-hebi have practically no proteolytic activity while the venoms of Hyappoda, Ao-hebi and Taiwan-habu have respectively a fairly remarkable proteolytic activity. This fact is also very interesting and attractive

when we consider that the former belong to "Haemorrhagic venom" and the latter belong to "Neuro-toxin." Anyhow, further investigation must be made in detail before any definite conclusion can be drawn on the specificities of these groups of venoms classified i.e. "Haemorrhagic venom" and "Neuro-toxin."



Comparison of the venoms of various kinds of snakes, from the standpoint of their activation power upon the proteolytic activity of trypsin. Curves were drawn only for convenience sake to make easier the quick comparison. (cf. Table 13, p. 134)

SUMMARY

1. The activation power of the snake venoms upon proteolytic activity of dried pig's pancreas (30%) glycerine extract was investigated. In the main part of this experiment, the venom of snake, Taiwan-habu (*Trimeresurus mucrosuquamatus*, CANTOR) was used, though we employed for comparison, the venoms of various kinds of snakes, such as Hyappo-da (*Agkistrodon actus*, GÜNTHER), Ao-hebi (*Trimeresurus graminens*, SHAW), Taiwan-kobura (*Naja naja atra*, CANTOR) and Amagasa-hebi (*Bungarus multicinctus*, BLYTH) etc.
2. As the substrate casein, sometimes gelatin and Witte's peptone were used, and determination of proteolytic action was carried out by the alcohol titration method described in detail in our previous paper.⁽⁷⁾
3. On the time required for full activation of trypsin. A fairly remarkable activation was made even when contact of trypsin with

the venom was made for so short an interval as 10 minutes, and the time required for full activation of trypsin was 30-180 minutes at 40°, according to C_V and C_E (cf. Symbols, p. 110) employed. (Figs. 1, 2 and 3, pp. 112 and 113).

4. On the relation between the amount of venom and that of trypsin for the full activation of the latter.

Any proportional relation between them was not found at all. Anyhow, the venom in such a low concentration as 0.04 mg had sufficient power to make the full activation of pancreatic trypsin in its concentration such as $C_E=5.3$ mg. (Figs. 4 and 5, pp. 114 and 115).

5. Stability of the venom from the standpoint of its activation power upon the proteolytic activity of trypsin.

The stability at each pH was in the following order.

pH 2.0 < pH 6.1 < pH 8.0 > pH 10.1 (Fig. 6, p. 117)

The thermal resistance at each temperature was in the following order. 40° < 50° < 55° < 56° - 80° (Fig. 7, p. 119)

6. Activation power of the venom upon the proteolytic activity of trypsin was decreasing as the time of the auto-activation of 30 % glycerine extract of trypsin lengthened and the activation became practically zero when the time of the auto-activation reached 96 hours. (Fig. 8, p. 120)

7. No activation power of the venom was observed upon the proteolytic activity of the commercial trypsin preparations. (Table 9, p. 132)

The explanation regarding this was given on p. 121.

8. On the activation power of the venom upon the proteolytic activity of trypsin at varying pH with different substrates such as casein, gelatin and Witte's peptone.

The activation of the venom upon trypsin was most favourable at the pH zone 7-8.0 optimal for the activity of trypsin. (Figs. 9, 10 and 11, pp. 122 and 123)

9. Fig. 12 illustrates clearly the comparison of the venoms of various kinds of snakes, from the standpoint of their activation power upon the proteolytic activity of trypsin. Reference should also be made to explanations on p. 124.

TABLE 3.

On the time required for full activation of trypsin by the snake venom.

1. Activation tested for comparatively long time.

For activation:

 $C_E' = 2C_E = 10.6 \text{ mg}$, $C_V' = 2C_V$, $C_G = 18\%$,

Time of contact of trypsin and the venom is as noted below.

For digestion:

 $C_4 = 3\%$ of casein, $C_L = 5.3 \text{ mg}$, C_V is as noted below, $C_G = 9\%$,

 pH of each digestion mixture is 8.0 (at 40°)

 Digestion for 1 hour at 40° .

Time of contact of trypsin and venom in hours	X			
	$C_V = 0.04 \text{ mg}$	$C_V = 0.012 \text{ mg}$	$C_V = 0.008 \text{ mg}$	$C_V = 0$
0	0.60	0.52	0.44	0.45
2	2.29	2.12	1.29	0.82
6	2.16	1.98	1.74	1.00
12	2.12	2.01	1.76	1.08
24	2.07	1.94	1.62	1.17
48	1.98	1.77	1.44	1.16
72	1.91	1.68	1.29	1.11

TABLE 4.

On the time required for full activation of trypsin by the snake venom.

2. Activation tested for comparatively short time.

For activation:

 $C_1' = 2C_1$, $C_V' = 2C_V$, $C_1 = 18\%$,

Time of contact of trypsin and the venom is as noted below.

For digestion:

 $C_3 = 3\%$ of casein, C_L and C_V are as noted below, $C_G = 9\%$,

 pH of digestion mixture is 8.0 (at 40°)

 Digestion for 1 hour at 40°

C_L mg	Time of contact of trypsin and venom in minutes	X						
		$C_V = 0.04 \text{ mg}$	$C_V = 0.02 \text{ mg}$	$C_V = 0.012 \text{ mg}$	$C_V = 0.008 \text{ mg}$	$C_V = 0.004 \text{ mg}$	$C_V = 0.0008 \text{ mg}$	$C_V = 0$
5.3	0	0.76	0.54	0.44	0.46	0.41	0.38	0.41
	10	1.94	1.60	1.34	1.20	0.87	0.63	0.42
	30	2.14	2.03	1.96	1.67	1.33	0.87	0.51
	60	2.19	2.15	2.10	2.00	1.72	1.11	0.65

C _E mg	Time of contact of trypsin and venom in minutes	X						
		C _V = 0.04 mg	C _V = 0.02 mg	C _V = 0.012 mg	C _V = 0.008 mg	C _V = 0.004 mg	C _V = 0.0008 mg	C _V =0
5.3	120	2.18	2.16	2.14	2.07	1.93	1.28	0.83
	180	—	—	2.14	2.08	1.99	1.48	0.92
	240	—	—	2.05	2.06	1.96	1.52	0.94
2.7	0	—	—	0.37	0.29	0.32	—	0.30
	10	—	—	1.07	0.86	0.65	—	0.34
	30	—	—	1.60	1.29	1.04	—	0.36
	60	—	—	1.68	1.58	1.39	—	0.46
	120	—	—	1.68	1.67	1.55	—	0.53
	180	—	—	1.67	1.66	1.53	—	0.58
	240	—	—	1.69	1.65	1.50	—	0.62

TABLE 5.

Relation between the amount of venom and that of trypsin

For activation:

$C_L' = 2C_E$, $C_V' = 2C_V$, $C_G = 18\%$,

Activation for 1 hour at 40°

For digestion:

$C_4 = 3\%$ of casein, C_E and C_V are as noted below, $C_G = 9\%$,

pH of each digestion mixture is 8.0 (at 40°)

Digestion for 1 hour at 40°.

C _V mg	C _E = 5.3 mg	C _E = 2.7 mg	C _E = 1.3 mg	C _E = 0.65 mg	C _E = 0.26 mg	C _E = 0.13 mg	C _L = 0
0	0.66	0.33	0.21	0.16	0.08	0.04	0
0.0003	1.57	1.14	0.76	0.50	0.15	0.05	0
0.0024	1.91	1.40	1.06	0.56	0.22	0.12	0
0.004	1.99	1.53	1.12	0.66	0.24	0.13	0
0.008	2.13	1.73	1.19	0.78	0.28	0.12	0
0.012	2.23	1.73	1.29	0.81	0.36	0.14	0
0.02	2.24	1.82	1.43	0.90	0.47	0.17	0
0.04	2.24	1.92	1.51	1.14	0.51	0.24	0.03

Cv mg	X						
	C _E =5.3 mg	C _E =2.7 mg	C _E =1.3 mg	C _E =0.65 mg	C _E =0.26 mg	C _E =0.13 mg	C _E =0
0.1	2.23	1.91	1.56	1.19	0.69	0.35	0.10
0.5	2.20	1.90	1.63	1.31	0.92	0.61	0.24
1.0	2.19	1.89	1.67	1.35	0.97	0.73	0.36
2.0	2.20	1.90	1.67	1.44	1.04	0.83	0.43

TABLE 6.

pH-stability-relation of the venom from the standpoint of its activation power upon the proteolytic activity of trypsin.

For stability:

Each venom water solution (Taiwan-habu) was regulated to pH as noted in part "a" of pH in the following table, and kept standing for varying lengths of time.

For activation:

C_I' = 2C_I = 10.6 mg, C_V' = 2C_V = 0.08 mg, C_I = 18%,

pH as described in part "b" of pH in the following table.

Each mixture of enzyme and venom solution was kept for 1 hour at 40°.

For digestion:

C_V 3% of casein, C_E 5.3 mg, C_V = 0.04 mg, C_I 9%,

pH of the digestion mixture was all regulated to 8.0 (at 40°), as shown in part "c" of pH in the following table.

For control experiment:

Experiment was carried out in the same way as above except that, for stability experiment, the venom water solution was kept standing at its natural pH in a refrigerating chamber at 0°.

Time of pH-stability in hours	X													
	pH			pH			pH			pH			control experiment	
	(a)	(b)	(c)	(a)	(b)	(c)	a)	b)	c)	(a)	(b)	(c)	with	without
	2.0	6.2	8.0	6.1	6.2	8.0	8.0	8.0	8.0	10.0	8.0	8.0	venom	venom
0	0.68			2.20			2.29			2.16			2.23	0.70
2	0.58			2.18			2.30			1.95			—	—
6	0.61			2.09			2.18			1.23			—	—
12	0.58			1.89			2.25			0.64			2.22	—
24	0.64			1.28			1.90			0.49			2.27	—
36	0.65			0.82			1.56			0.56			2.22	—
48	0.68			0.84			1.22			0.53			2.25	—
60	0.59			0.73			1.03			0.54			2.26	—
72	0.70			0.80			0.87			0.56			2.31	—
96	0.72			0.77			0.75			0.57			2.25	—

TABLE 7.

Thermal resistance of the venom water solution from the standpoint of its activation power upon the proteolytic activity of trypsin.

For thermal resistance:

0.01% venom (Taiwan-habu) water solution was kept standing for 0-120 minutes at various temperature, 40°-80°.

For activation:

$C_E' = 2C_E = 10.6$ mg, $C_V' = 2C_V = 0.08$ mg, $C_G = 18\%$,
Activation for 1 hour at 40°.

For digestion:

$C_s = 3\%$ of casein solution, $C_E = 5.3$ mg, $C_V = 0.04$ mg, $C_G = 9\%$,
pH of digestion mixture is 8.0 (at 40°)

For control experiment:

$C_s = 3\%$ of casein, $C_E = 5.3$ mg, $C_V = 0$,
pH of digestion mixture is 8.0.

Digestion for 1 hour at 40° after enzyme solution was kept standing for 1 hour at 40°.

Time of thermal resistance in minutes	X						Control ex- periment
	40'	50'	55'	60'	70°	80°	
0	2.24	—	—	—	—	—	0.67
10	2.24	2.04	0.99	0.72	0.66	0.69	—
30	2.23	1.79	0.77	0.64	—	—	—
60	2.19	1.36	0.71	0.66	—	—	—
120	2.21	1.06	0.72	0.65	0.66	0.64	—

TABLE 8.

The activation power of the venom upon the proteolytic activity of trypsin of pig's pancreas which was subjected to its spontaneous auto-activation for varying lengths of time.

For auto-activation of trypsin:

1% pancreas glycerine (30%) solution was kept standing at 40° for varying lengths of time.

For activation:

$C_E' = 2C_E = 10.6$ mg, $C_V' = 2C_V = 0.08$ mg, $C_G = 18\%$.
Activation for 1 hour at 40°.

For digestion:

$C_s = 3\%$ of casein, $C_E = 5.3$ mg, $C_V = 0.04$ mg, $C_G = 9\%$.
pH of digestion mixture is 8.0.
Digestion for 1 hour at 40°.

Time (hours) subjected to auto-activation of trypsin	X	
	With venom	Without venom
0	2.20	0.55
2	2.10	0.85
6	1.88	1.00
12	1.81	1.02
24	1.53	1.05
48	1.19	1.03
72	1.14	1.05
96	1.02	1.00
120	0.93	0.91
144	0.81	0.83

TABLE 9.

The activation power of the venom upon the proteolytic activity of commercial trypsin.

For activation :

$C_E' = 2C_T = 24.4$ mg, $C_V' = 2C_V = 0.08$ mg, $C_G = 18\%$,

Activation for 1 hour at 40° .

For digestion :

$C_S = 3\%$ of casein, $C_E = 12.2$ mg, $C_V = 0.04$ mg, $C_G = 9\%$.

pH of digestion mixture is 8.0.

Digestion for 1 hour at 40° .

Commercial trypsin employed	Water content of preparation	C_T	X	
			With venom	Without venom
Merck	18.78%	12.2 mg	1.33	1.34
Grubler	18.52%	12.2 mg	0.52	0.53

TABLE 10.

On the activation power of the venom upon the proteolytic activity of trypsin at varying pH.

(1) Casein-splitting.

For activation:

$C_E' = 2C_E = 2.4$ mg, $C_V' = 2C_V = 0.024$ mg, $C_G = 8.4\%$,

Activation for 1 hour at 40° .

For digestion:

$C_s = 3\%$ of casein, $C_E = 1.2$ mg $C_V = 0.012$ mg, $C_G = 4.2\%$.

pH of each digestion mixture is shown in the table.

Digestion for 1 hour at 40° .

pH before and after digestion	X	
	With venom	Without venom
6.2	1.15	0.23
6.5	1.29	0.24
6.8	1.37	0.29
7.4	1.40	0.26
7.7	1.43	0.28
8.0	1.35	0.20
8.3	1.24	0.19
8.6	1.10	0.18
8.9	1.02	0.18
9.2	0.77	0.13
9.5	0.62	0.12
9.8	0.53	0.12
10.1	0.42	0.13

TABLE 11.

On the activation power of the venom upon the proteolytic activity of trypsin at varying pH

(2) Gelatin splitting.

For activation:

$C_E' = 2C_E = 2.4$ mg, $C_V' = 2C_V = 0.024$ mg, $C_G = 8.4\%$,

Activation for 1 hour at 40° .

For digestion :

$C_E=3\%$ of gelatin, $C_E=1.2$ mg, $C_V=0.012$ mg, $C_G=4.2\%$.

pH of each digestion mixture is shown in the table.

Digestion for 1 hour at 40° .

pH before and after digestion	X	
	With venom	Without venom
3.0	0	0
4.0	0.02	0
5.0	0.06	0.02
6.0	0.28	0.05
6.4	0.48	0.07
7.0	0.55	0.07
7.4	0.64	0.13
7.7	0.66	0.11
8.0	0.67	0.14
8.3	0.61	0.09
8.6	0.61	0.06
8.9	0.40	0.10
9.2	0.26	0.09
9.5	0.26	0.06
9.8	0.18	0.07
10.1	0.07	0.07

TABLE 12.

On the activation power of the venom upon the proteolytic activity of trypsin at varying pH.

(3 Witte's peptone splitting.

For activation :

$C_{E'}=2C_E=2.4$ mg, $C_{V'}=2C_V=0.024$ mg, $C_G=8.4\%$,

Activation for 1 hour at 40° .

For digestion :

$C_E=1.5\%$ of Witte's peptone, $C_E=1.2$ mg, $C_V=0.012$ mg, $C_G=4.2\%$.

pH of each digestion mixture is shown in the table.

Digestion for 1 hour at 40° .

pH before and after digestion	X	
	With venom	Without venom
4.0	0.09	0.04
5.0	0.08	0.04
6.0	0.32	0.10
6.4	0.48	0.20
7.0	0.67	0.24
7.4	0.82	0.30
7.7	0.81	0.30
8.0	0.75	0.28
8.3	0.64	0.23
8.6	0.57	0.20
8.9	0.51	0.23
9.2	0.44	0.24
9.5	0.43	0.20
9.8	0.34	0.24
10.1	0.30	0.26

TABLE 13.

Comparison of the venoms of various kinds of snakes, from the standpoint of their activation power upon the proteolytic activity of trypsin.

Part A.

Snake venoms C _V =0.1 mg	X	
	With pancreas trypsin (C _E =5.3 mg)	Without pancreas trypsin
Taiwan-kobura	0.59	0.02
Amagasa-hebi	1.67	0.04
Hyappo-da	2.16	0.47
Ao-hebi	2.07	0.21
Taiwan-habu	2.29	0.32
Control experiment (Without any venom)	0.66	—

Part B.

Snake venoms C _V =0.04 mg	X	
	With pancreas trypsin (C _E =5.3 mg)	Without pancreas trypsin
Taiwan-kobura	0.65	0
Amagasa-hebi	0.87	0.03
Hyappo-da	1.32	0.07
Ao-hebi	1.39	0
Taiwan-habu	2.29	0

For activation:

C_E'=2C_E - 10.6 mg, C_V' 2C_V, C_G=18%, Activation for 1 hour at 40°.

For digestion:

C_s=3% of casein, C_E=5.3 mg, C_V=0.1 mg (in part A and C_V 0.04 mg (in part B), C_G=9%.

Digestion for 1 hour at 40°.

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ENZYME CHEMICAL INVESTIGATION OF FORMOSAN SNAKE VENOMS, III.*

STUDIES ON THE ACTIVATION OF PEPTIDASE BY THE SNAKE VENOMS

**Part I, On the Activation of Peptidase by the Venom of Snake,
Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)]†**

(with 7 Text-Figures,

Yoshio TSUCHIYA

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* [Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ., Formosa, Japan. Vol. IX. No. 5, December, 1935].

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INTRODUCTION

The investigations on the specific effect (activation or inhibition) of the snake venoms upon the activity of enzyme was considered to be one of the most interesting lines of great significance, of the enzyme chemical investigations of the snake venoms.

Starting from this consideration, the author searched for the literature but could not find any investigations except those of DELEZENNE (1902)¹ and LAUNOY (1902)² in which the specific effect of some snake venoms upon the inactive pancreatic juice, was somewhat investigated.

Therefore, in our Laboratory, the following fundamental investigations were undertaken on somewhat a large scale, i. e.,

- (1) On the specific effect of snake venoms upon trypsin.
- (2) On the specific effect of snake venoms upon peptidase.
- (3) On the specific effect of snake venoms upon other enzymes.

The author was allotted a part (2) of this work and carried out detailed investigations on this subject, and discovered several interesting facts which are of great significance on the study of peptidase. These will be communicated in the successive papers.

The present investigation was made on the effect of the venom of the snake which is called "Taiwanhabu,"—one of the Formosan venomous snakes, upon the LG-cleavage of dipeptidase existing in the

extract of dried liver of tortoise, as well as that of fresh or dried intestinal mucous membrane of pig.

The results of which are summarized on p. 153.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO., for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI, for his assistance in the collection of snake venom, and in the preparation of peptides used in the present investigation.

EXPERIMENTAL PART

A. Preparation of Enzyme Extracts and Venom solutions.

1. *Enzyme materials.*

Dried liver of tortoise and dried or fresh intestinal mucous membrane of pig were used in the present investigation. For the preparation of dried materials, the method of "acetone-ether treatment" according to WILLSTÄTTER and WALDSCHMIDT-LEITZ^{11, 8} was employed.

2. *Dried venom.*

The original venom, which was a somewhat viscid fluid of pale yellowish tint, was collected from Taiwanhabu according to YAMAGUCHI's method,¹¹ and dried as rapidly as possible under suction in a vacuum desiccator over calcium chloride.

3. *Enzyme extracts.*

The enzyme materials were mixed with varying concentrations of glycerine solution, and the mixture was ground in a porcelain mortar. Each mixture was then centrifuged and filtered. Clear filtrate thus obtained was used for each experiment.

Data with regard to the preparations are given in table II.

4. *Venom solutions.*

Dried venom was mixed either with 30 % glycerine solution or with water by thorough shaking.

Each solution was then centrifuged and filtered. Clear filtrate thus obtained was used for each experiment.

Data with regard to the preparations are given in table II.

B. Preparation of Substrate Buffer Solutions.

1. *Substrates.*

Dipeptides used in the present investigation were all racemic. They were prepared according to FISCHER's method⁵ and analysed for carboxyl groups, amino groups and total nitrogen. The peptides were recrystallized at least once.

2. *Substrate buffer solutions.*

Substrate buffer solutions were prepared in exactly the same way as that of LINDERSTRÖM-LANG and SATO⁶; i. e., 0.2 mol. peptide (mono-amino and mono-carboxyl) + 0.15 mol. ammonia + 0.25 mol. ammonium chloride in 1 litre, pH 8.0.

C. Determination of Enzyme Activity.

The determination of enzyme activity was carried out according to the semi-micro titration method as devised and modified by LINDERSTRÖM-LANG and SATO.⁶ This method was based upon the same principle as the alcohol titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ.¹¹ For the sake of reference, the author has noted only important points of this method in the following:

The total volume of the digestion mixture is 5 cc, from which each 2 cc is pipetted off before and after the digestion under the given conditions, and the increase of carboxyl groups per 2 cc of the mixture is measured by the above titration method. (cf. p. 141).

Throughout all the digestion experiments, the same conditions were kept as follows:

Substrate concentration = 0.1 mol.,

Glycerine concentration = 15 %,

pH = 8.0 (ammonia-ammonium chloride buffer),

Digestion for 1 hour at 40°.

For the determination of pH-activity-curves, the pH of each digestion mixture was regulated by the addition of ammonia or acetic acid to the above substrate-buffer solution, making the total volume 5 cc. (cf. p. 142 and also the literature⁹)

D. Process of Digestion with and without the Snake Venom.

1. General process.

Each digestion experiment was carried out in a small double digestion vessel provided with a cork stopper, which was devised according to LINDERSTRÖM-LANG⁵ and SATO.⁷

For the experiment without venom, 2.5 cc of substrate buffer solution was introduced in one limb of a digestion vessel, and a required amount of enzyme solution with an amount of 30 % glycerine solution sufficient to make the volume 2.5 cc was introduced in another limb of the vessel.

For the experiment with venom, 2.5 cc of substrate buffer solution was introduced in one limb of a digestion vessel, and the same amount of enzyme solution as in the former case with an amount of 30 % glycerine solution sufficient to make the volume after the addition of venom solution 2.5 cc was introduced.

After the addition of a required amount of the venom solution, each digestion vessel was dipped by turns up to the neck in a water thermostat at 40°, and warmed for precisely ten minutes.

Then the contents of both limbs of each digestion vessel were well mixed by careful shaking for about one minute, and in the following 30 seconds, 2 cc were carefully measured off into a good pipette.

After one minute and 30 seconds, the 2 cc were poured into a small 50 cc ERLÉNMEYER's flask containing 10 cc of 96 % alcohol, the mixture was shaken well, and the digestion vessel was replaced in the thermostat at 40°. After the lapse of the time required for digestion, which was measured from the moment of the mixing of the liquids in the two limbs, 2 cc were again removed with the same pipette (after being rinsed with ca. 0.5 cc of experimental liquid) and mixed in the same way with 10 cc of alcohol.

The alcohol mixtures with an addition of 0.4 cc of 0.5% thymolphthalein solution (90% alcoholic) were then titrated with n/20 alcoholic potassium hydroxide solution, the titration being first carried to a rather strong bluish-green colour, then 20 cc of 96% alcohol was

added, whereupon the titration was continued till the first bluish shade appeared. A micro-burette calibrated into 1/50 cc was used.

The difference of the quantities of $n/20$ KOH solution required before and after digestion, corresponds to the increase of carboxyl groups formed per 2 cc of digestion liquid under the experimental conditions, and that of the increase of two sets of digestion with and without venom, corresponds to the magnitude of activation.

2. Process for testing the time of contact of venom and enzyme for maximum activation of dipeptidase.

In one limb of a large double digestion vessel was placed several times the amount of venom solution required for each 5 cc of digestion mixture, and several times the amount of enzyme extract required for each 5 cc of digestion mixture in another limb of the vessel. Each vessel was dipped in a water thermostat at 40°.

After the vessel had been kept standing for ten minutes, the contents of both limbs were well mixed by careful shaking for one minute, and then replaced in the thermostat at 40°.

For the control experiment, 30% glycerine solution was used instead of venom solution.

After standing for varying lengths of time, which was measured from the moment of mixing the contents of both limbs of each digestion vessel, each 2.5 cc was carefully measured off into a good pipette, and introduced into one limb of a small digestion vessel, in another limb of which was placed 2.5 cc of substrate buffer solution, which had been warmed in a thermostat at 40° for ten minutes.

Then each digestion experiment was carried out immediately in exactly the same way as noted in the general process (1).

3. Process for the determination of pH-activity-curves.

In a similar way to the general process (1), in one limb of a small digestion vessel, were introduced 2.5 cc of substrate buffer solution and acetic acid or ammonia solution, which was sufficient to make the varying pH of each digestion mixture in the case of making

the total volume 5 cc by mixing with the enzyme, 1 % venom, and 30% glycerine solution which had been introduced in another limb of the vessel.

TABLE I.

pH-regulation of the substrate buffer solution of pH 8.0.

In order to regulate the pH of the digestion mixture as indicated below, 2.5 cc of the substrate buffer solution of pH 8.0 (cf. p. 140) were mixed with each 1.0 cc of 30% glycerine solution (No. 1-9) containing acetic acid or ammonia as in the following table.

No.	pH obtained at 40°.	cc of the reagents to be made up to 50 cc with 88% glycerine 17.1 g) and with water.			
		acetic acid		ammonia	
		1n	2n	2n	4n
1	6.05	0	9.08	0	0
2	6.6	0	7.99	0	0
3	6.95	0	7.20	0	0
4	7.2	0	6.00	0	0
5	7.7	5.75	0	0	0
6	8.0	0	0	0	0
7	8.4	0	0	6.45	0
8	8.8	0	0	0	8.10
9	9.1	0	0	0	18.75

The amount of acetic acid or ammonia to be added is given on table I.

With regard to the amounts of enzyme, venom, and glycerine to be added, reference should be made to table II (p. 146) and the tables of each experimental result. The venom solution was kept standing for 10 minutes at 40° before mixing the contents of both limbs of the vessel.

The further process was the same as (1) (cf. p. 141)

4. *Process for the determination of stability of the activation power of the venom.*

a) *On the determination of stability in aqua as well as in 30% glycerine solution at their natural pH.*

Five times the amount of 1 % aqueous or 30% glycerine solution required for each 5 cc of digestion mixture, were taken in a large test tube and kept standing in a water thermostat at 40° for varying lengths of time.

After each standing, 0.4 cc of each venom solution was pipetted off and immediately subjected to each digestion experiment, in a similar manner to that noted in the section of general process on p. 141.

As the control, the corresponding digestion experiment was carried out as usual without the venom.

b) *On the determination of stability at varying pH.*

In one limb of a small digestion vessel were placed 4 cc of 1% venom glycerine (30%) solution and in another limb of the vessel were introduced 2 cc of 30% glycerine solution (a) or 1.4 cc of n/100 ammonia glycerine (30%) solution plus 0.6 cc of 30% glycerine solution (b) or 1.4 cc of n/50 ammonia glycerine (30%) solution plus 0.6 cc of 30% glycerine solution (c), respectively.

Each vessel was dipped by turns up to the neck in a water thermostat at 40°, and warmed for 10 minutes. Then the contents of both limbs of each digestion vessel were mixed under thorough shaking for about one minute, and the vessel was replaced in the thermostat.

The pH of each mixture was, (a) 5.7 (=natural pH), (b) 7.0 and (c) 8.5.

After the lapse of the time of standing, which was measured from the moment of mixing by a stop watch, 1.2 cc of each mixture was pipetted off and immediately subjected to each digestion experiment. (cf. the general process on p. 141)

As the control, the corresponding digestion experiment was carried out as usual without the venom.

The pH of each digestion mixture was examined and found to be 8.0 without any deviation according to varying cases.

Each pH of venom solutions regulated as well as of the

digests before and after digestion was determined at 40° by colorimetric method.

E. Symbols.

The symbols used in the present investigation are summarized below :

- 1) AG, LG, etc., = Leucylglycine, alanylglycine, etc., otherwise as noted in tables VI (p. 156) and XVI (p. 160).
- 2) X = Number of carboxyl groups formed during the digestion, expressed in cc of $n/20$ KOH per 2 cc of the digestion mixture; X_{LG} for the cleavage of leucylglycine, X for the cleavages of peptides noted in tables VI and XVI, and X_{auto} for the autolysis of the mixture of enzyme extract and venom solution.
- 3) C_E = Enzyme concentration, expressed in mg. of original dried enzyme material corresponding to the amount of enzyme extract used per 2 cc of the digestion mixture.
- 4) C_V = Venom concentration, expressed in mg. of original dried venom of snake corresponding to the amount of venom solution used per 2 cc of the digestion mixture.

F. Enzyme Extracts and Venom Solutions Used.

Table II contains a survey of the enzyme extracts and the venom solutions used in the present investigation and the particular data relating to their preparations.

G. Experimental Results.

Relation between the LG-cleavage and the time of standing of the enzyme-venom mixture before digestion experiment.

The enzyme extract of dried liver of tortoise and the venom solution were mixed together before digestion experiment, and the

TABLE II.

Survey of the enzyme extracts and the venom solutions used.

No. of enzyme extract or venom solution	Material used		g of material used	Total volume of mixture cc	Conc. of solvent	pH of enzyme extract or venom solution	
						C	Q
Enzyme extract	I* ¹	Dried liver of tortoise	0.2399	25	30%G	6.3	6.37
	II* ¹		0.7197	"	"	—	—
	III* ¹		0.4798	35	"	—	—
	IV* ²	Fresh	15	100	60%G	6.0	5.96
	V* ³	Dried intestinal mucous membrane of pig	0.5	25	60%G	6.1	6.18
	VI* ³		"	"	"	—	—
Venom solution	I* ⁴	Dried venom	0.1	10	30%G	5.7	5.66
	II* ⁴		"	"	W	—	—

Note:—

- *1. The dried material (water content 8.80%) was prepared 11/9 1934, which was the same one as No. 3 used in a previous paper⁹. In each case, the enzyme extracts were newly prepared just before use.
- *2. The fresh material (water content 80.87%) and the enzyme extract were prepared 9/11 1934, and the extract was kept standing in an ice-chamber at 0° before use.
- *3. The dried material (water content 5.83%) was prepared 9/11 1934, and extract V was prepared 9/11 1934, and kept standing in an ice-chamber at 0° before use. The extract VI was prepared 17/11 1934, from the same material, and used immediately.
- *4. In each case, the solutions were newly prepared just before use.
G=glycerine, W=water. C=colorimetric, Q=quinhydrinic.

mixture was kept standing in a water-thermostat at 40° for varying lengths of time. Then the LG-cleavage of dipeptidase was determined respectively. According to this result (cf. Table III, p. 155; Fig. I), it was found that the LG-cleavage of dipeptidase was remarkably activated by the venom of Taiwanhabu, but the magnitude of this

activation was dependent upon different lengths of time of standing, i. e., the longer the time of standing, the less the activation power.

And the activation disappears within a comparatively short time of standing,—such as 100 minutes.

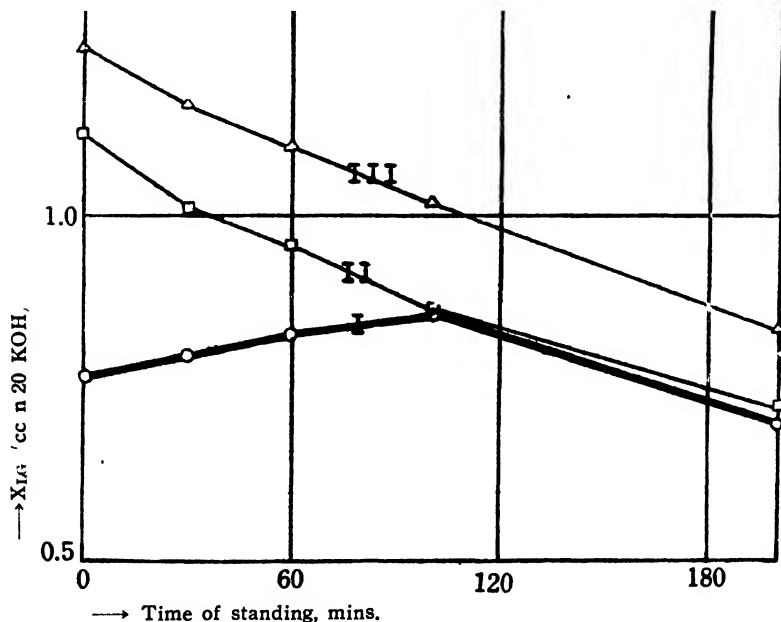
The same fact was also observed in the case of the extract of fresh and dried intestinal mucous membrane of pig, (cf. Tables IV, p. 155 and V, p. 156; Fig. II).

It should be noticed here that the disappearance of the activation for the fresh and dried intestinal mucous membrane of pig was more rapid than for the dried liver of tortoise.

FIG. I.

Curves illustrating the activation of dipeptidase of 30% glycerine extract of dried liver of tortoise when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time.

The curves correspond to the figures in table III.



Curves: I, $C_E=3.50$, $C_V=0$.
 II, $C_E=3.50$, $C_V=2.0$.
 III, $C_E=3.50$, $C_V=4.0$.

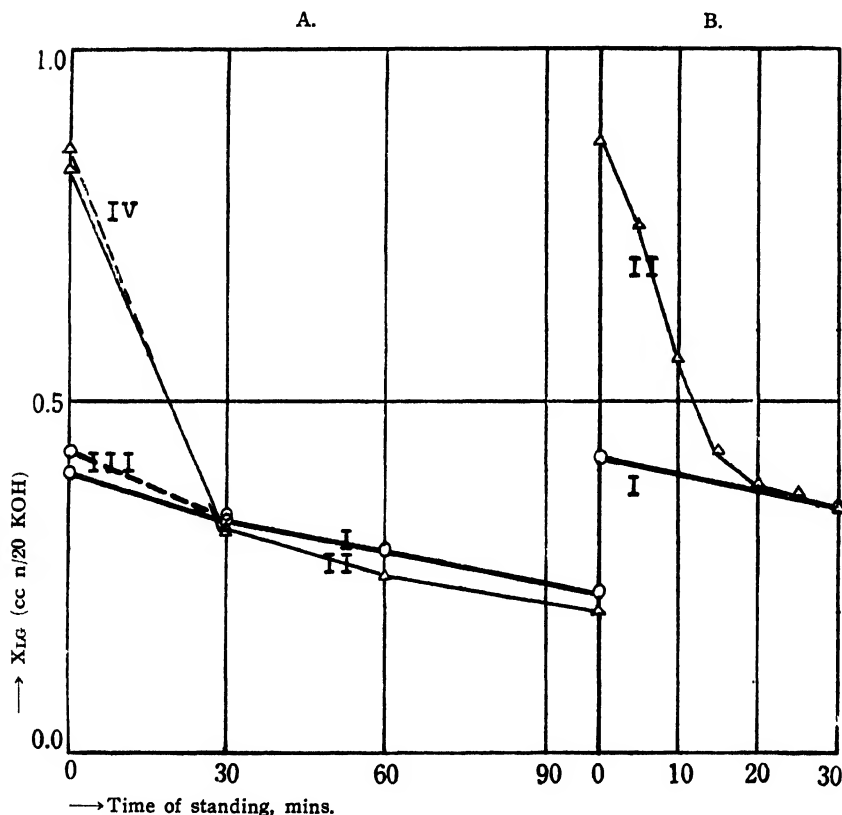
FIG. II

- A. Curves illustrating the activation of dipeptidase of 60% glycerine extract of *fresh*¹ and *dried*² intestinal mucous membrane of pig when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time.

The curves correspond to the figures in table IV.

- B. Curves illustrating the activation of dipeptidase of 60% glycerine extract of *fresh*³ intestinal mucous membrane of pig when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time.

The curves correspond to the figures in table V.



Curves: I, *Fresh*,¹ $C_E=0.82$, $C_V=0$.
 II, *Fresh*,¹ $C_E=0.82$, $C_V=2.4$
 III, *Dried*,² $C_E=2.51$, $C_V=0$.
 IV, *Dried*,² $C_E=2.51$, $C_V=2.4$.

Curves: I, *Fresh*,³ $C_E=0.82$, $C_V=0$.
 II, *Fresh*,³ $C_E=0.82$, $C_V=2.4$.

As control experiment for the activation, the following two factors were examined: 1) the activity of dipeptidase of the venom itself.

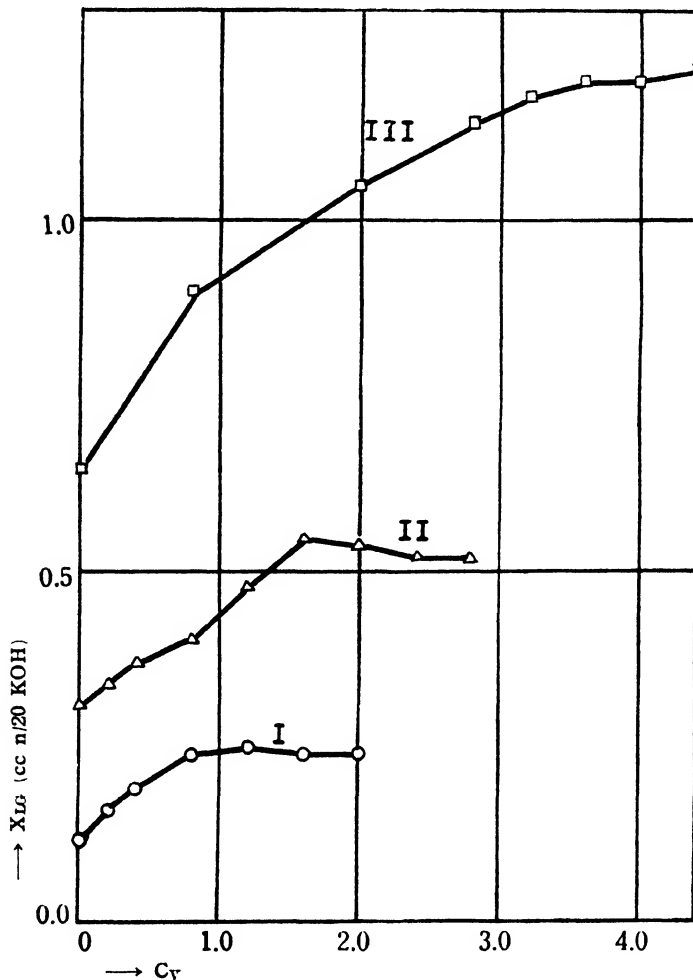
2) the autolysis of the mixture of each enzyme extract and the venom solution.

But the author observed neither a perceptible dipeptidase activity

FIG. III.

Curves illustrating the activation of dipeptidase of 30% glycerine extract of dried liver of tortoise when the extract was mixed with varying quantities of the venom of snake. Curves also showing the C_V required for the maximum activation corresponding to varying C_E used.

The curves correspond to the figures in table X.



Curves: I, $C_E=0.88$; II, $C_E=1.75$; III, $C_E=3.50$.

of the venom itself nor a perceptible autolysis of the mixture of each enzyme extract and the venom solution, thus showing that no influence can be given upon the activation by the two examined factors. (cf. Tables VI, VII, VIII and IX, p. 156-157).

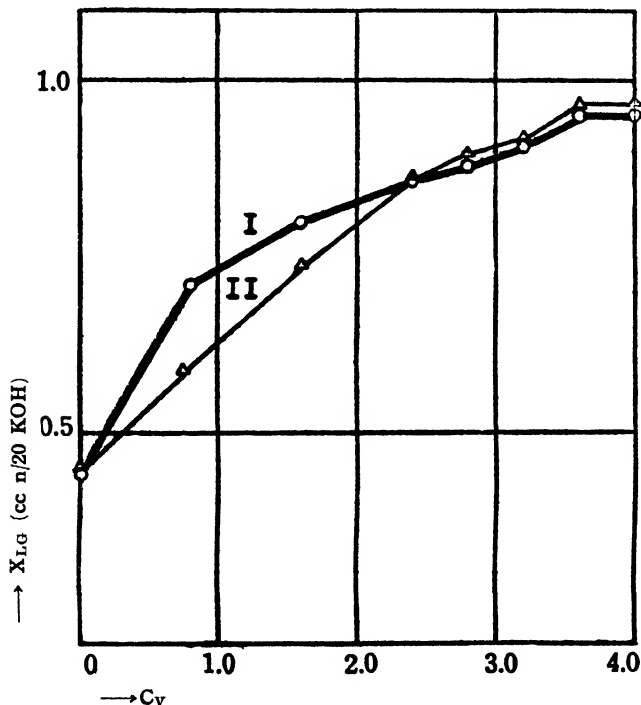
The quantity of venom solution required for the maximum activation.

Without standing the mixture of the enzyme extract and the venom solution before experiment,* (i. e., mixing the enzyme, venom, and substrate solutions precisely at the beginning of the digestion

FIG. IV.

Curves illustrating the activation of dipeptidase of 60% glycerine extract of fresh and dried intestinal mucous membrane of pig when the extract was mixed with varying quantities of the venom of snake. Curves also showing the C_V required for the maximum activation corresponding to C_E used.

The curves correspond to the figures in table XI.



Curves: I, Fresh, $C_E=0.82$; II, Dried, $C_E=2.51$.

* All experiments described later on were carried out in the same way as noted here.

experiment),—it was determined how much venom is required for the maximum activation of LG-cleavage of dipeptidase, when the extract of dried liver of tortoise and fresh or dried intestinal mucous membrane of pig were used.

According to this result (cf. Tables X and XI, p. 157–158; Fig. III and IV), it was ascertained in each case that the maximum activation for a definite quantity of the enzyme extract was caused by a definite quantity of venom solution.

And generally speaking, the quantity of venom solution required for the maximum activation was proportional to the quantity of enzyme extract.

A similar relation was also shown in the case of enterokinase according to WALDSCHMIDT-LEITZ.¹²

Stability of the venom solution observed from the standpoint of its activation power upon the cleavage of leucylglycine.

The venom solution at its natural pH or at varying pH which was regulated with dilute ammonia, was kept standing in a water-thermostat at 40° for varying lengths of time.. Then the activation power of the venom which was thus kept standing, was measured upon the LG-cleavage by dipeptidase of the extract of dried liver of tortoise.

Judging from this result (cf. Tables XII and XIII, p. 158; Fig. V), the stability of the venom solution at its natural pH was somewhat less in aqua than in 30% glycerine concentration, and in the latter case, at pH 8.5 than at pH 5.7 (=natural pH) and 7.0.

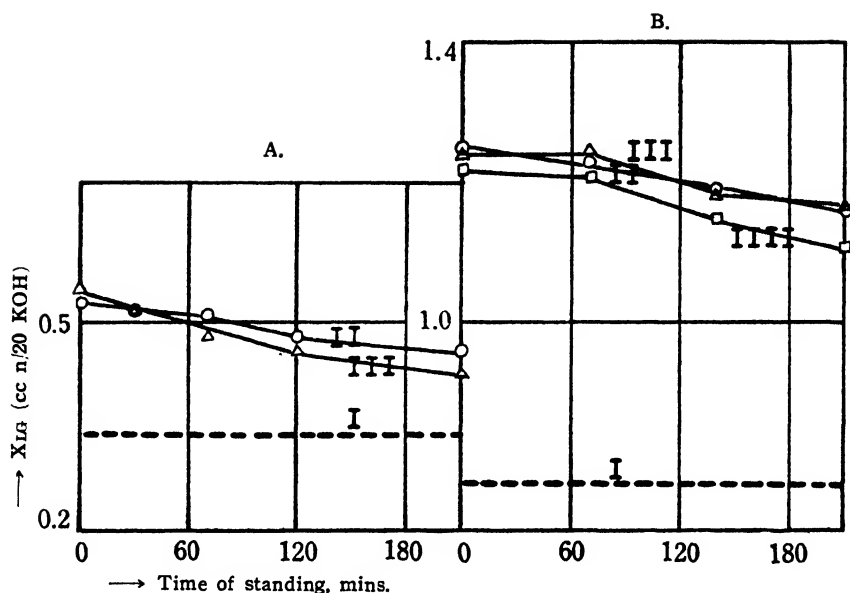
FIG. V.

- A. Curves illustrating the stability of 30% glycerine or aqueous solution of the venom of snake at its natural pH and at 40° as observed from the standpoint of its activation power upon the dipeptidase of 30% glycerine extract of dried liver of tortoise.

The curves correspond to the figures in table XII.

- B. Curves illustrating the stability of the venom of snake in 30% glycerine concentration at varying pH and at 40° as observed from the standpoint of its activation power upon the dipeptidase of 30% glycerine extract of dried liver of tortoise.

The curves correspond to the figures in table XIII.



Curves: I, $C_E=1.75$, $C_V=0$.
 II, $C_E=1.75$, $C_V=1.6$, 30% G.
 III, $C_E=1.75$, $C_V=1.6$, Water.

Curves: I, $C_E=3.50$, $C_V=0$.
 II, $C_E=3.50$, $C_V=3.2$, pH=5.7.
 III, $C_E=3.50$, $C_V=3.2$, pH=7.0.
 IV, $C_E=3.50$, $C_V=3.2$, pH=8.5.

However, generally speaking, the venom solutions can be said to be comparatively stable.

Comparison between the pH-activity-curves of LG-cleavage of dipeptidase, with and without the addition of the venom of snake.

The pH-activity-curves of LG-cleavage, one with and the other without venom, were obtained and shown in Fig. VI and VII. (cf. Tables XIV and XV, p. 159).

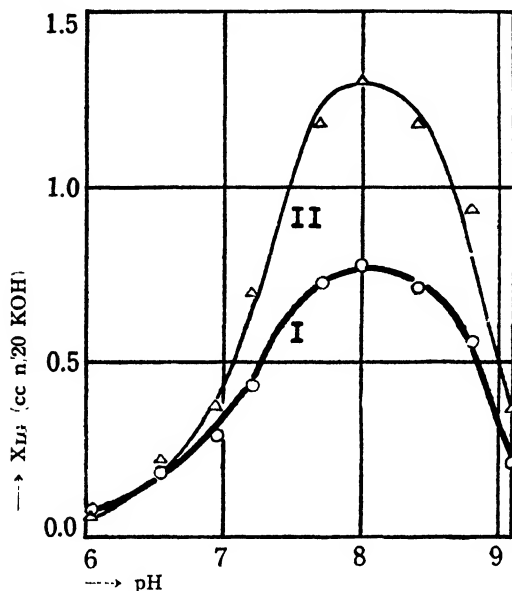
These were in three cases, i. e., in the case of using the extract of dried liver of tortoise and in the case of using the extract of fresh or dried intestinal mucous membrane of pig.

From these figures, a marked activation power of the venom was clearly observed throughout the pH-range in each case and sharp optimum activation at the optimum pH or pH-zone.

FIG. VI

Comparison between the pH-activity-curves of dipeptidase of 30% glycerine extract of dried liver of tortoise with and without the addition of the venom of snake.

The curves correspond to the figures in table XIV.



Curves: I, $C_E = 3.50$, $C_V = 0$; II, $C_E = 3.50$, $C_V = 3.2$.

ages of leucylglycine, glycylleucine, and glycylphenylalanine, while rather weak upon the cleavage of valylglycine, and none upon the cleavages of glycylglycine and alanylglycine under the given conditions.

SUMMARY

- 1) The venom of snake (Taiwanhabu) activated remarkably the LG-cleavage of dipeptidase of the extract of dried liver of tortoise as well as that of fresh or dried intestinal mucous membrane of pig.
- 2) This activation phenomenon was dependent upon the lengths of time of contact of the enzyme extract and the venom solution; namely, the longer the time of standing of the mixture, the less the power of activation of the enzyme (dipeptidase). This relation was somewhat different in its degree according to the enzyme materials used.

Activation power of the venom of snake upon the cleavages of various kinds of dipeptides.

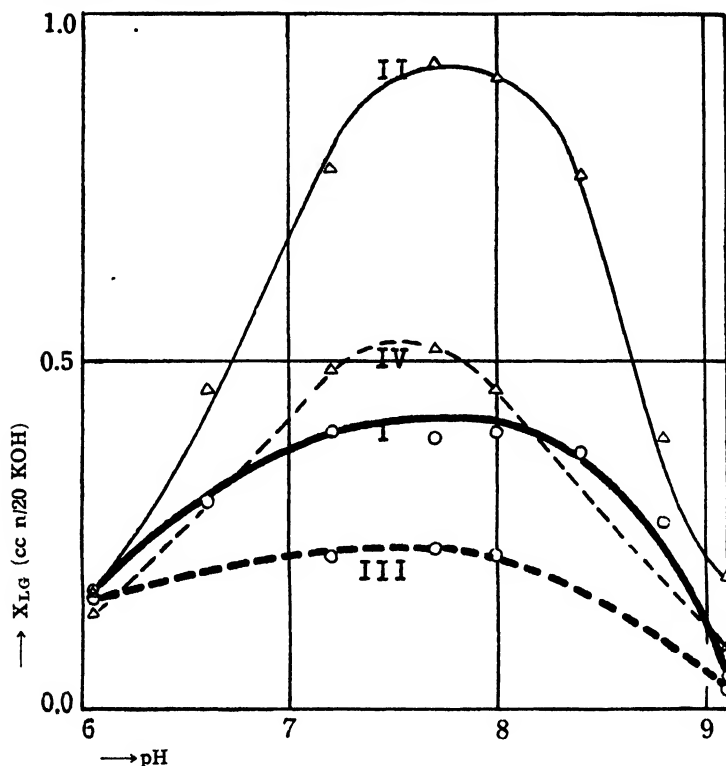
With the extract of dried liver of tortoise, a series of experiments was carried out for finding out whether the venom exerts the same activation phenomenon upon the cleavages of different kinds of dipeptides as upon that of leucylglycine.

According to this experiment (cf. Table XVI, p. 160), a very interesting fact was discovered that the activation of the venom was found to be strong upon the cleav-

FIG. VIII.

Comparison between the pH-activity-curves of dipeptidase of 60% glycerine extract of *fresh*¹ and *dried*² intestinal mucous membrane of pig with and without the addition of the venom of snake.

The curves correspond to the figures in table XV.



Curves: I, *Fresh*,¹ $C_E=0.82$, $C_V=0$. II, *Fresh*,¹ $C_E=0.82$, $C_V=4.0$.
 III, *Dried*,² $C_E=1.88$, $C_V=0$. IV, *Dried*,² $C_E=1.88$, $C_V=4.0$.

3) This activation phenomenon was brought about to the maximum by a definite quantity of the venom for a definite quantity of the enzyme extract, and the quantity of the venom required for the maximum activation was nearly proportional to the quantity of enzyme extract used.

4) Generally speaking, the power of activation of the venom solutions was comparatively stable under the given conditions.

5) Comparing the two pH-activity-curves, with and without the

venom, the activation was clearly observed throughout all the pH-range and sharp optimum activation at optimum pH or pH-zone.

6) The activation of the venom was found to be strong upon the cleavage of leucylglycine, glycylleucine, and glycyphenylalanine, while rather weak upon the cleavage of valylglycine, and absent upon the cleavage of glycyglycine and alanylglycine under the given conditions.

TABLE III

Activation of dipeptidase of 30% glycerine extract of dried liver of tortoise when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time. Enzyme extract. I. Venom solution. I. $C_D=3.50$.

Time of standing mins.	X_{LG}		
	$C_V = 0$	$C_V = 2.0$	$C_V = 4.0$
0	0.77	1.12	1.24
30	0.80	1.01	1.16
60	0.83	0.96	1.10
100	0.86	0.87	1.02
200	0.70	0.73	0.84

TABLE IV.

Activation of dipeptidase of 60% glycerine extract of fresh and dried intestinal mucous membrane of pig when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time.

Enzyme extract. IV and V. Venom solution. I. $C_F=0.82$ for enzyme extract IV and $C_L=2.51$ for enzyme extract V.

Time of standing, mins.	X_{IG}			
	Enzyme extract IV		Enzyme extract V	
	$C_V = 0$	$C_V = 2.4$	$C_V = 0$	$C_V = 2.4$
0	0.40	0.83	0.43	0.86
30	0.33	0.32	0.34	0.31
60	0.29	0.25	—	—
100	0.23	0.20	—	—

TABLE V.

Activation of dipeptidase of 60% glycerine extract of fresh intestinal mucous membrane of pig when the extract was mixed with the venom of snake and kept standing in 30% glycerine concentration at 40° for varying lengths of time. Enzyme extract. IV. Venom solution. I. $C_E=0.82$.

Time of standing, mins.	X_{LG}	
	$C_V=0$	$C_V=2.4$
0	0.42	0.87
5	—	0.75
10	—	0.56
15	—	0.43
20	—	0.38
25	—	0.37
30	0.35	0.35

TABLE VI.

Cleavage of various peptides by dipeptidase of 30% glycerine solution of dried venom of snake. Venom solution. I., $C_V=3.2$.

Substrate (Symbol)	X
Leucylglycine (LG)	0.03
Glycylleucine (GL)	0.03
Glycylphenylalanine (GPh)	0.03
Valylglycine (VG)	0.00
Alanylleucine (AL)	0.00
Glycylglycine (GG)	0.00
Alanylglycine (AG)	0.05
Alanylvaline (AV)	0.02

TABLE VII.

Cleavage of leucylglycine by dipeptidase of 30% glycerine solution of dried venom of snake for varying lengths of time of digestion. Venom solution. I., $C_V=3.2$.

Time of digestion, hrs.	X_{LG}
1	0.03
2	0.05
3	0.09

TABLE VIII.

Test on the autolysis with the enzyme-venom-mixtures without substrate for varying lengths of time of digestion.

Enzyme extract. I, IV, and V., Venom solution. I.

Time of digestion, hrs. pH 8.0)	X _{auto}		
	Enzyme extract I	Enzyme extract IV	Enzyme extract V
	C _E =3.50, C _V =3.2	C _E =0.82, C _V =4.0	C _E =2.51, C _V =4.0
1	0.01	0.02	0.00
2	0.02	0.04	0.02
3	0.04	0.07	0.04

TABLE IX.

Test on the autolysis with the enzyme-venom-mixtures without substrate at varying pH in the digestion mixture.

Enzyme extract. I., Venom solution. I., Time of digestion 1 hour., C_E=3.50, C_V=3.2.

pH of the digestion mixture	X _{auto}
7.4	0.01
8.0	0.03
8.9	0.03

TABLE X.

Activation of dipeptidase of 30% glycerine extract of dried liver of tortoise when the extract was mixed with varying quantities of the venom of snake, showing the C_V required for the maximum activation corresponding to varying C_E used.

Enzyme extract. I., Venom solution. I.

C _V	X _{L(1)}		
	C _L =0.88	C _L =1.75	C _L =3.50
0	0.12	0.31	0.65
0.2	0.16	0.34	—
0.4	0.19	0.37	—
0.8	0.24	0.40	0.90
1.2	0.25	0.48	—
1.6	0.24	0.55	—
2.0	0.24	0.54	1.05
2.4	—	0.52	—
2.8	—	0.52	1.14
3.2	—	—	1.18
3.6	—	—	1.20
4.0	—	—	1.20
4.4	—	—	1.22

TABLE XI.

Activation of dipeptidase of 60% glycerine extract of fresh and dried intestinal mucous membrane of pig when the extract was mixed with varying quantities of the venom of snake, showing the C_V required for the maximum activation corresponding to C_E used.

Enzyme extract. IV and V., Venom solution. I., $C_E=0.82$ for enzyme extract IV and $C_E=2.51$ for enzyme extract V.

C_V	X_{LG}	
	Enzyme extract IV	Enzyme extract V
0	0.44	0.45
0.8	0.71	0.59
1.6	0.80	0.74
2.4	0.86	0.87
2.8	0.88	0.90
3.2	0.91	0.92
3.6	0.95	0.97
4.0	0.95	0.97

TABLE XII.

Stability of 30% glycerine or aqueous solution of the venom of snake at its natural pH and at 40° as observed from the standpoint of its activation power upon the dipeptidase of 30% glycerine extract of dried liver of tortoise.

Enzyme extract I., Venom solution I and II., $C_F=1.75$.

Time of standing, mins.	X_{LG}		
	$C_V=0$	Venom solution I	Venom solution II
		$C_V=1.6$	$C_V=1.6$
0	0.34	0.53	0.55
30	0.34	0.52	0.52
70	0.34	0.51	0.48
120	0.34	0.48	0.46
210	0.34	0.46	0.43

TABLE XIII.

Stability of the venom of snake in 30% glycerine concentration at varying pH and at 40° as observed from the standpoint of its activation power upon the dipeptidase of 30% glycerine extract of dried liver of tortoise.

Enzyme extract I., Venom solution I., $C_F=3.50$.

Time of standing, mins.	X_{LG}			
	$C_V=0$	$C_V=3.2$		
		pH=5.7	pH=7.0	pH=8.5
0	0.77	1.25	1.24	1.22
70	0.77	1.23	1.25	1.21
140	0.77	1.19	1.18	1.15
210	0.77	1.16	1.17	1.11

TABLE XIV.

Comparison between the pH-activity-relations of dipeptidase of 30% glycerine extract of dried liver of tortoise, with and without the addition of the venom of snake.

Enzyme extract III., Venom solution I., $C_E=3.50$.

pH of the digestion mixture	X_{LG}	
	$C_V=0$	$C_V=3.2$
6.05	0.08	0.07
6.6	0.19	0.22
6.95	0.29	0.38
7.2	0.43	0.70
7.7	0.73	1.19
8.0	0.78	1.31
8.4	0.71	1.18
8.8	0.56	0.91
9.1	0.22	0.37

TABLE XV.

Comparison between the pH-activity-relations of dipeptidase of 60% glycerine extract of fresh and dried intestinal mucous membrane of pig, with and without the addition of the venom of snake.

Enzyme extract IV and VI., Venom solution I., $C_V=0.82$ for enzyme extract IV and $C_E=1.88$ for enzyme extract VI.

pH of the digestion mixture	X_{LG}			
	Enzyme extract IV		Enzyme extract VI	
	$C_V=0$	$C_V=4.0$	$C_V=0$	$C_V=4.0$
6.05	0.17	0.17	0.16	0.14
6.6	0.30	0.46	—	—
7.2	0.40	0.78	0.22	0.49
7.7	0.39	0.93	0.23	0.52
8.0	0.40	0.91	0.22	0.46
8.4	0.37	0.77	—	—
8.8	0.27	0.39	—	—
9.1	0.05	0.19	0.03	0.09

TABLE XVI.

Activation power of the venom of snake upon the cleavages of various kinds of dipeptides by dipeptidase of 30% glycerine extract of dried liver of tortoise.

Enzyme extract II, Venom solution I.

Substrate (Symbol)	C _R	X		Activation power	
		C _V =0	C _V =3.2	Per cent of increase ¹	Relative rate of activation ²
Leucylglycine (LG)	3.50	0.84	1.32	57	+
Glycylleucine (GL)	3.50	0.80	1.12	40	+
Glycylphenylalanine (GPh)	5.25	0.65	1.01	55	+
Valylglycine (VG)	1.17	0.65	0.82	26	+?
Alanylleucine (AL)	10.50	0.40	0.49		—
Glycylglycine (GG)	3.50	0.57	0.53		—
Alanylglycine (AG)	0.35	0.55	0.59		—
Alanylvaline (AV)	10.50	0.03	0.03		—

Note: 1. For convenience sake, per cent of increase of activation was calculated as follows:

$$\frac{\times (\text{with venom}) - \times (\text{without venom})}{\times (\text{without venom})} \cdot 100$$

2. Symbol + denotes a marked activation power,—denotes practically no activation power.

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PART II.

On the Activation of Dipeptidase by the Venom of Snake, Taiwan-habu [*Trimeresurus mucrosquamatus* (CANTOR)] upon the Cleavage of Alanylglycine and Leucylglycine with Special Reference to the Test of Activation with Dipeptidase Extracts from Different Origins.

'with 3 Text-Figures

Yoshio TSUCHIYA

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INTRODUCTION

In a previous paper,¹⁰ the author reported on the influence of the venom of Taiwanhabu upon the peptidase extracts prepared from the liver of tortoise and the intestinal mucous membrane of pig for the cleavage of peptides and found that there was a marked activation of dipeptidase by the venom for the cleavage of some peptides such as leucylglycine, glycylleucine, and glycylphenylalanine, while practically no perceptible activation was observed for the cleavage of the other peptides, such as glycylglycine and alanylglycine. Among these data, the point which attracted the author's closest attention was the fact that the cleavage of leucylglycine was markedly activated while the cleavage of alanylglycine suffered no perceptible activation.

Thus, the author considered that, continuing the research on this point, it might be possible to get yet another key to lock out the question which lies between the enzymatic cleavage of leucylglycine and alanylglycine, though the specific differences which existed between them were already investigated in detail by K. LINDERSTRÖM-LANG,^{2,3} K. LINDERSTRÖM-LANG and Masakazu SATO,⁵ Masakazu SATO,^{6,7,8} Masakazu SATO, and the author,⁹ from the view points of enzyme stability, affinity between enzyme and substrate or pH-activity-curves, etc.

In the consideration above stated, the present work was intended to find out whether the same fact, as above indicated, exists upon the enzymatic cleavage of leucylglycine and alanylglycine when the extracts of several kinds of enzyme materials prepared from natural sources (animals and plants) were widely used.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO, for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI, for his assistance in the collection of snake venom, and in the preparation of the peptides used in the present investigation.

EXPERIMENTAL PART

A. Preparation of Substrate Buffer Solutions.

The substrates and substrate stock solutions were prepared in exactly the same way as described in a previous paper.¹⁰

B. Preparation of Enzyme Materials and Dried Venom.

1. *Fresh enzyme materials of pig, snake, tortoise, zebu, carp and hens.*

The fresh enzyme materials were prepared from the fresh entrails, tissues or digestive organs of animals, by grinding into gruel-like condition in a meat mincer.

2. *Dried enzyme materials of the above animals.*

The dried enzyme materials were prepared from the above gruel-like fresh materials in the same method as that of "acetone-ether treatment" according to WILLSTÄTTER and WALDSCHMIDT-LEITZ.¹¹

3. *Fresh rice-sprouts.*

The seeds of rice were sprouted in a thermostat at 30° for 5 days, then the fresh sprouts were carefully separated from the seeds, and were ground in a mortar.

4. *Dried yeast.*

This material was prepared by the DAI NIPPON Brewery Co. Ltd., and is said to be used for the manufacture of "Ebios," i. e., vitamin-B-preparation.

5. *Takadiastase.*

This material was SANKYŌ's chemical, which is well known as a patented digestive.

6. *Dried venom.*

The dried venom was prepared in exactly the same way as that noted in a previous paper.¹⁰

C. Preparation of Enzyme Extracts and Venom Solutions.

These extracts and venom solutions were prepared from the above enzyme materials and dried venom in the same manner as

TABLE I
Survey of the enzyme extracts used.

Enzyme material			Enzyme extract used					Total volume of mixture cc	Conc. of solvent G %
No. of material	Kind of material	Date of preparation	No. of extract	Date of preparation	pH of enzyme extract		g of material used		
					C	Q			
1	Dried intestinal mucous membrane	10/2 1935	ID ₁	15/2 1935	6.1	6.18	0.5	25	60
2	"	"	ID ₂	3 3	—	—	"	"	30
3	Fresh liver	"	II	10/2 "	—	6.30	25	100	60
4	Fresh pancreas	"	III	" "	5.7	6.00	25	100	60
	Dried pancreas	"	IIID ₁	19 2 "	—	5.48	2.0	25	60
	"	"	IIID ₂	4 4 "	—	—	1.0	25	30
	"	"	IIID ₃	9 4 "	—	—	1.0	25	30
5	Fresh kidney	8 2 "	IV	8 2 "	6.4	6.37	30	100	60
6	Dried kidney	"	IVD	19 2 "	5.6	5.61	0 5	25	60
7	Fresh spleen	"	V	8 2 "	—	6.06	30	100	60
8	Dried spleen	"	VD	19 2 "	5.7	5.69	0.5	25	60
9	Fresh muscle	25/2 "	VI	25/2 "	—	6.14	25	51.5	60
10	Dried muscle	"	VII	28/2 "	6.2	6.35	5	50	30
11	Dried liver	"	VIII	27/2 "	6.2	6.23	0.5	25	30

12	Fresh muscle	25 2	VIII	25 2	—	6.06	25	55	60
13	Dried muscle	" "	VIII _D	28 2	6.1	6.19	5	50	30
14	" "	21/11 1932	VIII _D	20 2	—	6.32	2	25	30
15	Fresh liver	25/2 1935	IX	25 2	—	6.19	10	25	30
16	Dried liver	" "	XD ₁	27 2	6.1	6.26	0.25	25	30
17	" "	11/9 1934	XD ₂	24 2	6.3	6.37	0.48	50	30
18	" "	" "	XD ₃	1 3	—	—	0.48	50	30
	" "	28/11 1932	XD ₄	20 2	—	5.97	1.5	25	30
19	Fresh muscle	6 3 1935	XI	6 3 1935	—	6.00	20	50	60
20	Fresh liver	" "	XII	" "	—	5.85	12	50	60
21	Fresh liver of hens	6/3 1935	XIII	6/3 1935	—	6.21	16	50	60
22	Dried liver of zebu	4/2 1933	XIV _D	21/2 1935	—	5.81	2.5	25	30
23	Fresh sprout (稻)	12/2 1935	XV	12/2 1935	—	5.88	20	50	60
24	" " (芋)	" "	XVI	" "	—	5.83	15	50	60
25	Dried yeast	—	XVII _D	12/3 1935	—	6.10	30	150	30
26	Takadiastase 'SANKYŌ's'	—	XVII _D	6/2 1935	—	6.68	1.0	25	30

C=Colorimetric. Q=Quinhydrionic, G=Glycerine.

that previously stated,¹⁰ with a few exceptions which are described below :

1) The extract in the case of dried yeast was the autolysate, which was prepared by the same principle as the method of GRASSMANN¹; i. e.,

30 g of the dried yeast were homogeneously mixed with 116 cc of water and the paste-like fluid (Ca. 80% water content) was quickly liquefied by the addition of 15 cc of acetic ester under good stirring. After about 10 minutes, 150 cc of water were added to the resulting mixture, and the mixture was left to stand at ordinary temperature.

The acid produced during the standing was neutralized with a continuous addition of 0.5 n ammonia solution.

After 1.5 hours, the mixture was centrifuged and the supernatant liquid was discarded. The precipitate was then well washed with 600 cc of water. To the acetic ester free precipitate thus obtained, 51.1 g of conc. glycerine (88%) and some water were added making the total volume 150 cc. The mixture was subjected to autolysis for 24 hours, then centrifuged and filtered. The filtrate thus obtained was used for the purpose.

2) The 1 or 2% solution of the venom was prepared, the glycerine concentration of which was 30%.

The 2% solution was used in the case of dried yeast and the 1% solution was used in all other cases, and they were newly prepared just before use in each case.

D. Enzyme Materials and Enzyme Extracts Used.

Table I contains a survey of the enzyme extracts used in the present investigation and the particular data relating to their preparations,

Table II contains a survey of the water content of enzyme materials employed, the data of which are to be taken for the calculation of C_D .

TABLE II.

Survey of the water content of enzyme materials shown in table I.

The C_E was calculated, taking these data in this table into consideration, except some extracts; i.e., the extracts of fresh sprouts of rice (Nos. 23 and 24 in table I).

Enzyme material		
No.	Kinds of materials	Water content %
1	Dried intestinal mucous membrane	9.0 73.7 60.5 10.9 78.3 8.8 79.2 8.7
2	Fresh liver	
3	Fresh pancreas	
4	Dried pancreas	
5	Fresh kidney	
6	Dried kidney	
7	Fresh spleen	
8	Dried spleen	
9	Fresh muscle	76.3 14.0 10.0
10	Dried muscle	
11	Dried liver	
12	Fresh muscle	82.7 12.7 8.8 69.8 11.8 8.8 8.8
13	Dried muscle	
14	Dried muscle	
15	Fresh liver	
16	Dried liver	
17	Dried liver	
18	Dried liver	
19	Fresh muscle	63.1 65.0
20	Fresh liver	
21	Fresh liver of hens	75.8
22	Dried liver of zebu	10.7
25	Dried yeast	5.2
26	Takadiastase (SANKYŌ's)	7.5

E. Determination of Enzyme Activity.

The determination of enzyme activity was carried out in the same way as that noted in a previous paper¹⁰; i. e., according to the semi-micro alcohol titration method as devised and modified by LINDERSTRÖM-LANG and SATO.⁴

With a few exceptions, which will be duly noted, the digestive conditions were kept as follows:

Substrate concentration=0.1 mol.,

Glycerine concentration=15%,

pH=8.0±0.05 (ammonia-ammonium chloride buffer)

Digestion for 1 hour at 40°.

In all cases using venom, the mixture of venom solution and substrate buffer solution was mixed with the enzyme extract precisely at the beginning of the digestion experiments. (cf. the process on p. 141 in a previous paper¹⁰).

F. Symbols.

- 1) X_{AG} X_{LG} X_{auto} . = Number of carboxyl groups formed during the digestion, expressed in cc of n/20 KOH per 2 cc of the digestion mixture; X_{AG} or X_{LG} for the cleavage of alanylglycine or leucylglycine, X_{auto} for the autolysis of the enzyme extract or the mixture of enzyme extract and venom solution in the case of using no substrate.
- 2) C_E . = Enzyme concentration, expressed in mg. of original dried enzyme material corresponding to the amount of enzyme extract, or expressed in cc of the original enzyme extract, used per 2 cc of the digestion mixture.
- 3) C_V . = Venom concentration. (see the previous paper¹⁰).
- 4) t . = Time of digestion, expressed in minutes.
- 5) Q . = $[X_{AG}]/[X_{LG}]$ where $[X_{AG}]$ and $[X_{LG}]$ express the X_{AG} and X_{LG} when $C_E=1$ and $t=60$.

- 6) K_{AG} , K_{LG} , K_{auto} . = Quotient of X_{AG} , X_{LG} and X_{auto} . per unit time (min) of digestion; i. e., $K_{AG} = X_{AG}/t$, $K_{LG} = X_{LG}/t$, and $K_{auto} = X_{auto}/t$.
- 7) A_{AG} , A_{LG} = Activation for the cleavage of alanylglycine or leucylglycine caused by the addition of the venom, expressed in % of X_{AG} or X_{LG} which was determined without venom. The calculation of these values was explained on p. 169.

G. Remarks on the Judgement of Activation.

On the judgement of the activation of the enzyme by the venom, care should be taken on the autolysis of the mixture of enzyme extract and venom solution without substrate, as well as on the enzymatic power by the action of the venom itself.

On the former, it was found from a certain experiment that the autolysis does not occur till the digestion of the substrate becomes complete and then, for the first time, the autolysis appears. (cf. Table VI and VII, p- 174; also Fig. I, p. 170).

Therefore, in the present investigation, the autolysis was neglected from the calculation of the activation. On the latter, consideration was taken for the calculation though the enzymatic power was generally very small. (see p. 171).

H. Experimental Results.

The experimental results are clearly given in tables III (p. 172) and VIII (p. 175). No further comment is necessary except with regard to the calculation of the A_{AG} and A_{LG} as well as with regard to the sign used in table III.

(1) A_{AG} and A_{LG} were calculated by means of the following formula :

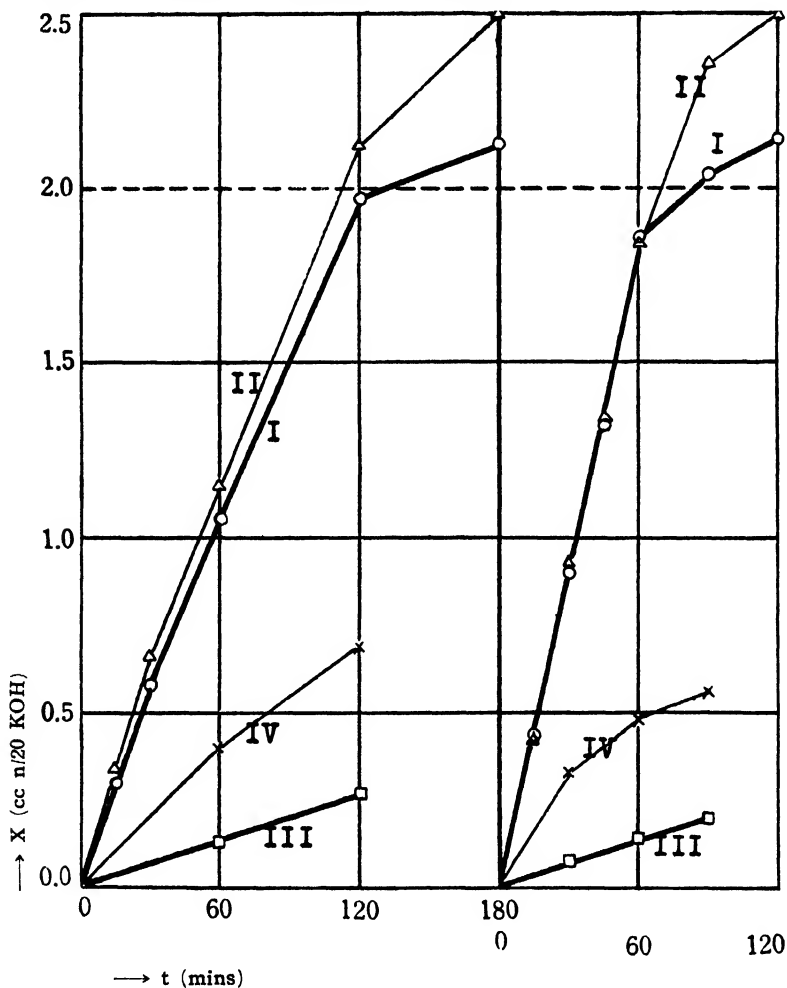
$$A_{AG} = \frac{X_{AG} \text{ (with venom)} - X_{AG} \text{ (without venom)} - \Delta X_{AG}}{X_{AG} \text{ (without venom)}} . 100$$

$$A_{LG} = \frac{X_{LG} \text{ (with venom)} - X_{LG} \text{ (without venom)} - \Delta X_{LG}}{X_{LG} \text{ (without venom)}} . 100$$

FIG. I.

Curves illustrating the relation between the cleavage of alanylglycine and leucylglycine by dipeptidase of 30% glycerine extract of dried pancreas of pig and the autolysis of enzyme extract as well as the autolysis of the mixture of enzyme extract and venom solution.

The curves correspond to the figures in tables VI and VII.



According to the above figures, the autolysis does not occur till the digestion of the substrate becomes complete, but after that it appears for the first time.

----- Total splitting line.

where ΔX_{AG} and ΔX_{LG} show the correction due to the small dipeptidase power of the venom itself when the digestion was carried out without other enzyme extract under the condition named, and were determined as follows:

ΔX_{AG}		ΔX_{LG}	
0.07		0.01	
0.05	0.06	0.03	0.02
0.06		0.02	

(2) In order to make easier a general survey of the activation, the data in table VIII (p. 175) are summarized in table III (p. 172) with signs of +, -, (-), etc.

These signs denote the degree of activation or inhibition, etc., and were defined as follows for convenience sake:

The sign + was used in the case of A_{AG} or A_{LG} being from +20 to +59%.

The sign ++ was used in the case of A_{AG} or A_{LG} being from +60 to +99%.

The others, + + +, + + + +, etc. were defined in a similar way to above examples.

The sign - was used in the case of A_{AG} or A_{LG} being from -20 to +19%.

The sign (-) was used in the case of A_{AG} or A_{LG} being from -60 to -21%.

SUMMARY

The activation by the venom of Taiwanhabu was widely tested upon the cleavage of leucylglycine and alanylglycine by dipeptidase of the extracts of several kinds of enzyme materials prepared from natural sources (animals and plants), and summarized in table III (p. 172).

With all the enzyme extracts, which had a Q-value varying from 0.5 to 9.2, a marked activation by the venom was observed upon the cleavage of leucylglycine, while no perceptible activation was noticed upon the cleavage of alanylglycine, though there were a few exceptions in some cases; i. e., in the case of using the extracts of pancreas of pig, muscle of carp, and rice-sprout, etc.

Finally, it may be noticed that such an interesting property of the venom has never been hitherto discovered as far as the author knows.

TABLE III.

Activation power of the venom of Taiwanhabu upon the cleavage of leucylglycine and alanylglycine by dipeptidase of the extracts of several kinds of enzyme materials prepared from natural sources.

The data correspond to the figures in table VIII.

Kinds of enzyme materials	Relative rate of activation*	
	Leucylglycine	Alanylglycine
Dried intestinal mucous membrane	##	—
Fresh liver	+	—
Fresh pancreas	—	—
Dried pancreas	—	—
Fresh kidney	##	—
Dried kidney	##	—
Fresh spleen	+	—
Dried spleen	+	—
Fresh muscle	+	—
Dried muscle	-?	—
Dried liver		—
Fresh muscle	+	—
Dried muscle	+	—
Fresh liver	+	—
Dried liver	—	—
Dried liver	+	—
Dried liver	+	—
Fresh muscle	—	—
Fresh liver	+	—
Fresh liver of hens	-?	—
Dried liver of zebu	+	—
Fresh sprout of rice	—	—
Dried yeast	+	(—)
Takadiastase (SANKYÔ's)	##	—

Note: * Symbols +, —, (—), etc. as noted on p. 171.

Values of A_{AG} or A_{LG} in parentheses in table VIII have not been taken in putting the signs down.

TABLE IV.

Relation between the amount of enzyme extract and the cleavage of leucylglycine and alanyl-glycine by dipeptidase of 30% glycerine extract of dried liver of tortoise.

Enzyme extract. XD.

C _E for X _{I.G} mg.	X _{LG}		K _{I.G}		A _{LG} C _V =4.0	C _L for X _{AG} mg.	X _{AG}		K _{AG}		A _{AG} C _V =4.0
	C _V =0	C _V =4.0	C _V =0	C _V =4.0			C _V =0	C _V =4.0	C _V =0	C _V =4.0	
1.75	0.35	0.61	0.0058	0.0102	+66	0.18	0.22	0.31	0.0037	0.0052	+14)
3.50	0.71	1.13	0.0118	0.0188	+56	0.35	0.49	0.58	0.0080	0.0097	+ 6
5.25	1.03	1.54	0.0172	0.0257	+48	0.53	0.70	0.79	0.0117	0.0132	+ 4
7.00	1.33	—	0.022	—	—	0.70	0.89	1.05	0.0148	0.0175	+11
8.75	1.54	—	0.0283	—	—	0.88	1.09	—	0.0182	—	—
						1.05	1.32	1.43	0.0220	0.0238	+ 4

TABLE V.

Relation between the time of digestion and the cleavage of leucylglycine by dipeptidase of 30% glycerine extract of dried intestinal mucous membrane of pig.

Enzyme extract. ID₁. C_D=1.21 mg.

t mins	X _{LG}			K _{LG}			A _{I.G}	
	C _V =0	C _V =0.8	C _V =4.0	C _V =0	C _V =0.8	C _V =4.0	C _V =0.8	C _V =4.0
30	0.16	0.18	0.30	0.0053	0.0060	0.0100	+13	+81)
60	0.30	0.35	0.63	0.0050	0.0058	0.0105	+17	+103
90	0.46	0.54	0.86	0.0051	0.0060	0.0096	+15	+ 80
120	0.63	0.71	1.14	0.0052	0.0059	0.0095	+11	+ 75
180	0.94	—	—	0.0052	—	—	—	—
240	1.24	—	—	0.0051	—	—	—	—

TABLE VI.

Relation between the time of digestion and the cleavage of alanylglycine
by dipeptidase of 30% glycerine extract of dried pancreas of pig.
Enzyme extract IIID₂. C_E=21.38 mg.

t mins	X _{AG}		K _{AG}		A _{AG} C _V =4.0	X _{auto.}		(b)-(a)	K _{auto.}	
	C _V =0	C _V =4.0	C _V =0	C _V =4.0		C _V =0 (a)	C _V =4.0 (b)		C _V =0	C _V =4.0
15	0.30	0.34	0.0200	0.0226	+ 8	—	—	—	—	—
30	0.58	0.66	0.0193	0.0220	+ 9	—	—	—	—	—
60	1.06	1.15	0.0177	0.0192	+ 3	0.14	0.40	0.26	0.0023	0.0067
120	1.97	2.12	0.0164	0.0177	—	0.28	0.69	0.41	0.0023	0.0058
180	2.12	2.50	0.0118	0.0139	—	—	—	—	—	—

TABLE VII.

Relation between the time of digestion and the cleavage of leucylglycine
by dipeptidase of 30% glycerine extract of dried pancreas of pig.
Enzyme extract. IIID₁. C_E=21.38 mg.

t mins	X _{LG}		K _{LG}		A _{LG}	X _{auto}		(b)-(a)	K _{auto}	
	C _V =0	C _V =4.0	C _V =0	C _V =4.0		C _V =0 (a)	C _V =4.0 (b)		C _V =0	C _V =4.0
15	0.44	0.44	0.0293	0.0293	0	—	—	—	—	—
30	0.90	0.93	0.0300	0.0310	+ 1	0.08	0.33	0.25	0.0027	0.0110
45	1.32	1.34	0.0293	0.0298	0	—	—	—	—	—
60	1.86	1.84	0.0310	0.0307	- 2	0.15	0.49	0.34	0.0025	0.0082
90	2.04	2.36	0.0227	0.0262	—	0.21	0.56	0.35	0.0023	0.0062
120	2.14	2.50	0.0178	0.0208	—	—	—	—	—	—

TABLE VIII.

The action of the venom of snake upon the cleavage of leucylglycine and alanyl-glycine by dipeptidase of the extracts of several kinds of enzyme materials prepared from natural sources.

No. of enzyme material	Kind of enzyme material	No. of enzyme extract.	C _E for X _{LG} mg.	X _{LG}		
				C _V =0	C _V =2.0	C _V =4.0
1	Dried intestinal mucous membrane	ID ₁	0.91	0.26	0.39	0.55
2	Fresh liver	II	1.31	0.58	0.67	0.75
3	Fresh pancreas	III	19.77	0.76	0.78	0.79
4	Dried pancreas	IIID ₁	5.94	0.56	0.58	0.57
5	Fresh kidney	IV	1.30	0.47	0.93	1.11
6	Dried kidney	IVD	1.22	0.26	0.37	0.57
7	Fresh spleen	V	1.25	0.33	0.55	0.67
8	Dried spleen	VD	2.61	0.37	0.53	0.61
9	Fresh muscle	VI	15.33	0.69	—	0.89
10	Dried muscle	VID	51.60	0.21	—	0.26
11	Dried liver	VIID	3.60	0.36	—	0.45
12	Fresh muscle	VIII	6.31	0.26	—	0.40
13	Dried muscle	VIID ₁	17.47	0.49	—	0.68
14	Dried muscle	VIID ₂	58.36	0.12	—	0.22
15	Fresh liver	IX	1.21	0.21	—	0.37
16	Dried liver	XD ₁	3.53	1.22	—	1.45
17	Dried liver	XD ₂	3.50	0.71	—	1.13
18	Dried liver	XD ₄	7.29	0.41	—	0.65
19	Fresh muscle	XI	29.54	0.55	—	0.55
20	Fresh liver	XII	16.82	0.33	—	0.48
21	Fresh liver of hens	XIII	1.55	0.79	—	0.90
22	Dried liver of zebu (黃牛)	XIVD	55.98	0.26 ^a	—	0.45 ^b
23	Fresh sprout (旭)	XV	1.0 ⁷	0.47 ^{4,8}	0.48 ^{4,8}	0.50 ^{4,8}
24	Fresh sprout (鳥糞)	XVI	1.0 ⁷	0.40 ^{4,8}	0.40 ^{4,8}	0.42 ^{4,8}
25	Dried yeast	XVIID	151.97	0.54 0.56 0.57	—	0.75 0.73 0.71
26	Takadiastase (SANKYŌ's)	XVIID	22.20 44.40	0.20 0.51 0.52 0.52	—	0.44 0.69 0.69 0.69

No. of enzyme material	A _{IG} ¹ Activation % C _V =4.0	C _E for X _{AG} mg.	X _{AG}			A _{AG} ¹ Activa- tion % C _V =4.0	C _E for X _{auto}	X _{auto} C _V =4.0	Q ²
			C _V =0	C _V =2.0	C _V =4.0				
1	+104	0.91	0.84	0.86	0.95	+ 6	—	—	3.2
2	+ 26	0.44	0.71	0.71	0.74	— 4	1.31	0.05	3.6
3	+ 1	19.77	0.78	0.79	0.84	0	19.77	0.32	1.0
4	— 1	7.13	0.58	0.58	0.62	— 3	5.94	0.21	0.9
5	+132	0.43	1.17	1.23	1.27	+ 3	1.30	0.05	7.5
6	+112	0.46	0.41	0.44	0.47	0	1.22	0.02	4.2
7	+ 97	0.42	0.82	0.86	0.90	+ 2	1.25	0.05	7.4
8	+ 59	0.61	0.23	0.26	0.26	(-13)	2.61	0.00	2.7
9	+ 26	3.07	0.59	—	0.65	0	15.33	0.06	4.3
10	(+ 14)	34.40	0.50	—	0.61	+ 10	51.60	0.06	3.6
11	+ 19	1.20	0.23	—	0.27	(- 9)	3.60	0.04	1.9
12	+ 46	2.10	0.62	—	0.68	0	6.31	0.04	7.2
13	+ 35	4.37	0.87	—	0.89	— 5	17.47	0.02 ^b	7.1
14	(+ 50)	29.18	0.25	—	0.35	(+12)	58.36	0.00	8.3
15	+ 67	0.24	0.32	—	0.37	— 3	1.21	0.02	7.7
16	(+ 17)	0.35	1.11	—	1.11	— 5	3.53	0.02	9.2
17	+ 56	0.35	0.49	—	0.58	+ 6	3.50	0.02	6.9
18	+ 54	2.92	0.52 ³	—	0.60	+10	7.29	0.01	6.3
19	— 4	19.69	0.76	—	0.80	— 3	29.54	0.10	2.1
20	+ 39	16.82	0.59	—	0.62	— 5	16.82	0.32	1.8
21	+ 11	0.52	0.87	—	1.00	+ 8	1.55	0.04	3.3
22	+ 50	37.32	0.28	—	0.34	0	37.32	0.02	4.8
23	— 2	0.5 ⁷	0.62 ^{4,8}	0.65 ^{4,8}	0.72 ^{4,8}	— 3	1.0 ⁷	0.10	2.6
24	— 5	0.5 ⁷	0.51 ^{4,8}	0.54 ^{4,8}	0.62 ^{4,8}	— 2	1.0 ⁷	0.09	2.6
25	+ 27	37.99	0.72 0.71 0.70	—	0.53 0.54 0.54	—32	151.97	0.02	5.1
26	+110	22.20	0.05	—	0.11	0	22.20	0.07	—
	+ 29	66.60	0.39	—	0.43	— 5	—	—	0.5

Note:—1. A_{AG} and A_{IG} were illustrated on pp. 169-171.

2. Q-value (=X_{AG}/X_{LG}) was calculated with an assumption that the enzyme activity was proportional to the time of digestion and the amount of enzyme in a certain range from which the data were taken. This assumption was confirmed with some enzyme extracts (cf. Table IV, p. 73, Fig. II p. 177; Table V, p. 73 Fig. III. p. 177).

3. 30 mins' digestion.

4. 120 mins' digestion.

5. 180 mins' digestion.

6. 240 mins' digestion.

7. expressed in cc.

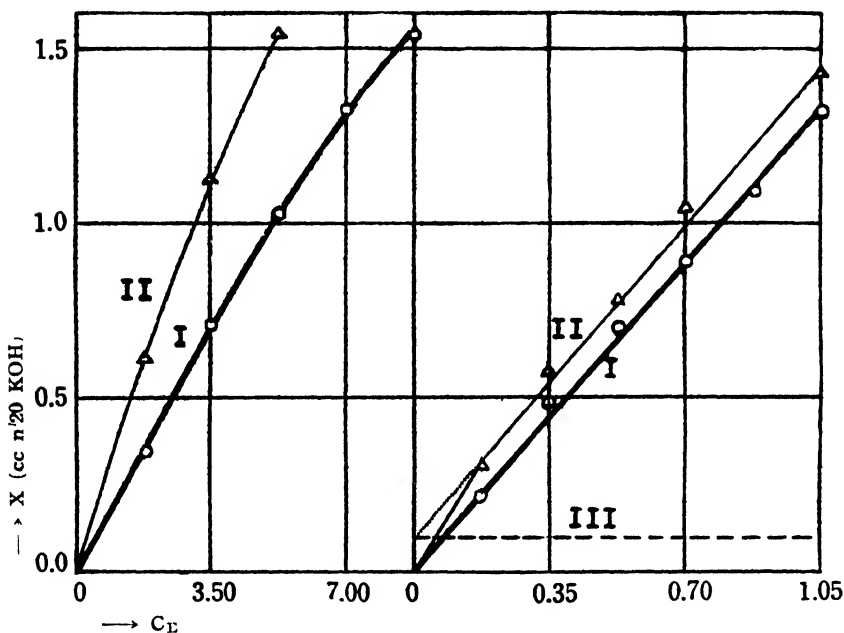
8. Glycerine concentration=21%.

Values of A_{AG} or A_{IG} in parentheses show the percentage of activation when the X_{AG} or X_{LG} was so small that the experimental error greatly influenced for the calculation, or so great that its value was not proportional to the time of digestion and the amount of enzyme.

FIG. II.

Curves illustrating the relation between the amount of enzyme extract and the cleavage of alanylglycine and leucylglycine by dipeptidase of 30% glycerine extract of dried liver of tortoise.

The curves correspond to the figures in table IV.



Curves: I, X_{LG} , $C_V=0$
 II, X_{LG} , $C_V=4.0$

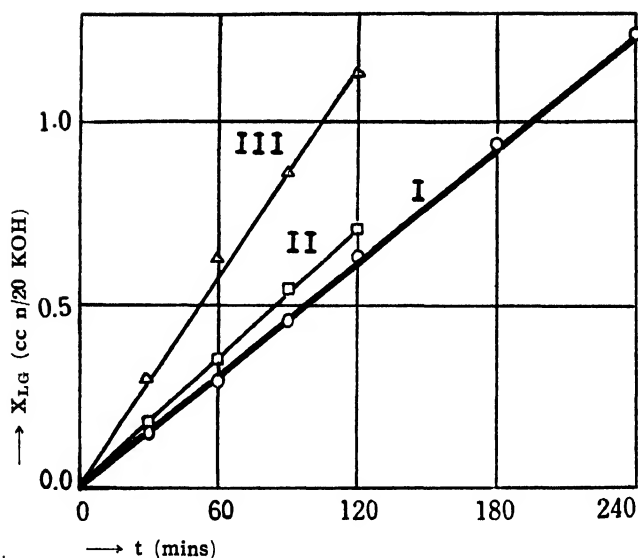
Curves: I, X_{AG} , $C_V=0$
 II, X_{AG} , $C_V=4.0$
 III, corresponds to the cleavage of alanylglycine due to the venom itself.

From the above figures, it is clear that the cleavages of alanylglycine and leucylglycine are proportional to the amount of enzyme extract.

FIG. III.

Curves illustrating the relation between the time of digestion and the cleavage of leucylglycine by dipeptidase of 30% glycerine extract of dried intestinal mucous membrane of pig.

The curves correspond to the figures in table V.



Curves: I, $C_E=1.21$, $C_V=0$
 II, $C_E=1.21$, $C_V=0.8$
 III, $C_E=1.21$, $C_V=4.0$

From the above figure, it is clear that the cleavage of leucylglycine is proportional to the time of digestion within about 60% of the total splitting.

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PART III.

On the Activation Power of the Venom of Taiwanhabu, [*Trimeresurus mucrosquamatus* (CANTOR)], upon the Dipeptidase of Purified Enzyme Extract.

(with 2 Text-Figures)

Yoshio TSUCHIYA

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INTRODUCTION

As stated in a previous paper,¹ in the cases of main kinds of enzyme materials tested, the venom of Taiwanhabu exercised a marked activation upon the enzymatic cleavage of leucylglycine while not upon the enzymatic cleavage of alanylglycine. There were also

a few exceptional cases of enzyme materials where no activation was observed upon both the peptides. In the present investigation, dried powder of the liver of tortoise or that of the kidney of pig was taken as a typical sample of the former, (proper example) and dried powder of the pancreas of pig as a typical sample of the latter (exceptional example) and test was made, with the venom of Taiwanhabu, and sometimes with the venom of Taiwankobura, on the question as to how such activation-relations arise when each extract of those enzyme materials is subjected to the purification process by the adsorption of $\text{Al}(\text{OH})_3$, C_7 . The results obtained are summarized on page 186 and clearly illustrated by table II (p. 188) as well as by Fig. I (p. 184) and II (p. 186).

The author wishes to express his sincere thanks to Prof Dr. Masakazu SATO for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI for his assistance in the collection of snake venoms, and in the preparation of peptides used in the present investigation.

EXPERIMENTAL PART

A. Preparation of Substrate Buffer Solutions.

The substrate and substrate buffer solutions were prepared in exactly the same way as described in previous papers.^{2, 3}

B. Preparation of Enzyme Materials.

As enzyme materials were used dried powder of entrails or digestive organs of pig and tortoise, the powder of which was prepared by the same procedure as that of "acetone-ether treatment" according to WILLSTÄTTER and WALDSCHMIDT-LEITZ.⁴

1. *Dried powder of the liver of tortoise.*

Two preparations, No. 2 and 5 in a previous paper¹ were used.

2. *Dried powder of the kidney and pancreas of pig.*

The same preparations as in a previous paper¹ were employed.

C. Preparation of Purified Enzyme Solution.

1. Original enzyme extract.

Original enzyme extracts were prepared from each dried powder of enzyme materials, by well extracting with 30% glycerine solution, etc. The details with regard to the preparation are given on table I. (p. 182).

2. Residual solution.

The pH of each enzyme extract was regulated to 5.0 with n/10 or n/100 acetic acid glycerine (30%) solution, and the extract thus regulated was subjected to the adsorption with aluminium hydroxide C γ .

The concentration of glycerine in the adsorption mixture was 30 % and the total volume of which was made twice that of original enzyme extract.

Proportions of each adsorption mixture were described in detail on each table.

The adsorption mixture was shaken well and left standing for ca. 5 minutes, centrifuged and filtered. Thus the residual solution was obtained.

3. Eludate.

The residue obtained as above was subjected to elution for ca. 30 minutes under occasional shaking with an equal volume of n/100 ammonia solution to that of original enzyme extract. Then the mixture was centrifuged and filtered. Thus the eludate was obtained.

D. Preparation of Dried Venoms.

Dried venoms of two kinds of snakes as noted below were prepared according to the method of YAMAGUCHI⁶:

- 1) The venom of Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)].

The appearance, etc. of the venom has already been described in a previous paper.²

- 2) The venom of Taiwankobura [*Naja naja atra* (CANTOR)].

The fresh venom was almost a colorless viscid fluid having an

appearance like saliva. But the dried venom, which was obtained by quick drying under suction in a vacuum desiccator, was a mass which had a somewhat crystalline appearance of a weak yellow tint.

Each of the dried venoms was crushed into fine powder in an agate mortar and the powder was preserved in a vacuum desiccator before use.

E. Preparation of Venom Solutions.

0.5, 1, or 2% venom glycerine (30%) solutions were prepared in a similar way to that noted in a previous paper.³

F. Preparation of Aluminium Hydroxide C₇.

Aluminium hydroxide C₇. was prepared 2/5 1935, according to the method of WILLSTÄTTER and his co-workers² and the water suspension contained 184.4 mg Al₂O₃ per 10 cc.

Before use, 1 vol. of original suspension was diluted with 1 vol. of 60% glycerine solution and 2 vols. of 30% glycerine solution.

Therefore the diluted suspension contained 46.1 mg Al₂O₃ per 10 cc and 30% glycerine.

TABLE I.
Survey of the enzyme materials and enzyme extracts.

Enzyme material used				Enzyme extract used		g of material used	Total volume of mixture cc	Conc. of solvent G %
No. of material	Kinds of materials	Date of preparation	Water content %	No. of extract	Date of preparation			
1	Dried kidney of pig	8/2 1935	8.8	Ia	12/6 1935	2	50	30
				Ib	10/7 1935		50	30
2	Dried liver of tortoise	11/9 1934	9.0	IIa	14/6 1935	2	50	30
				IIb	20/6 1935	4	100	30
3	Dried liver of tortoise	11/9 1934	10.9	IIc	14/6 1935	2	50	30
4	Dried pancreas of pig	31/5 1935	21.7	III	18/6 1935	4	50	30

G. Enzyme Materials and Enzyme Extracts Employed

In table I, is given a survey of enzyme materials and enzyme extracts employed together with particular data relating to their preparations. Each enzyme extract was newly prepared from dried enzyme materials just before use.

H. Determination of Enzyme Activity.

The determination of enzyme activity was carried out in the same way as that described in previous papers.^{2,3}

The digestive conditions were kept as follows, unless otherwise duly noted :

Substrate concentration=0.1 mol.,

Glycerine concentration=15%.

pH=8.0±0.05 (ammonia-ammonium chloride buffer),

Digestion for 60 mins at 40°.

At the digestion experiment, each component of original enzyme extract, residual solution, and eludate was taken in such an amount that each enzyme activity becomes as equal as possible to one another.

With regard to the process of digestion with and without the venom, reference should be made to p. 141 in a previous paper.²

I. On the Expression of the Purity of Enzyme Solution.

In the present investigation, the purity of enzyme solution was expressed by the ratio of each enzyme activity to its dried matter contained.

The purity of the residual solution as well as that of eludate were compared with that of original enzyme extract, assuming the purity of the latter being 1.

The purity of each enzyme solution thus expressed was shown on table V (p. 190), VIII (p. 192) and XI (p. 193).

Generally speaking, the purity of each enzyme solution judged from the standpoint of the above expression, was in the following order :

Eludate > Original enzyme extract > Residual solution,
i. e., it should be regarded as highest in the case of eludate while regarded as lowest in the case of residual solution.

J. Symbols.

The symbols used in the present investigation are the same as those previously noted.^{2, 3}

K. Experimental Results.

*On the activation of the venom upon the LG- and AG-cleavages by
dipeptidase of the original enzyme extract,
residual solution, and eludate.*

For the purpose of this experiment, the venom of Taiwanhabu and Taiwankobura were used and as enzyme materials the dried liver of tortoise, or dried kidney and pancreas of pig were employed.

1) When the dried kidney of pig was used as enzyme material.

a) When the venom of Taiwanhabu was employed.

As shown in tables III and IV (p. 189), it was observed that the activation degree upon the cleavage of leucylglycine was highest in the case of using the eludate, and lowest in the case of using the residual solution, while practically no perceptible activation was observed upon the cleavage of alanyl-glycine for each enzyme solution.

b) When the venom of Taiwankobura was employed.

The same relation as above stated can be thoroughly recognized also in Fig. I and tables XIV and XV (p. 195), in which varying amounts of Taiwankobura were used.

2) When the dried liver of tortoise was used as enzyme material, and the venom of Taiwanhabu was employed.

FIG. I.

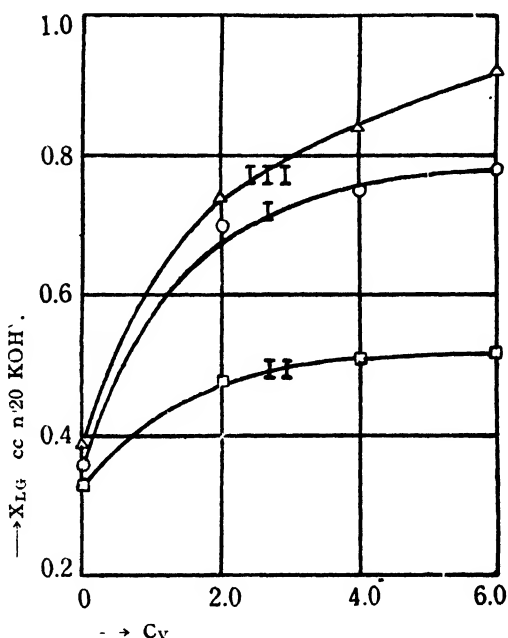
Curves illustrating the activation power of various amounts of the venom of Taiwankobura upon the LG-cleavage by dipeptidase of the original enzyme extract, residual solution and eludate obtained from the dried kidney of pig.

The curves correspond to the figures in table XIV.

According to the figures in tables VI and VII (pp. 190-191), the same tendency of activation upon the LG-cleavage could generally be found also in this case.

3) When the dried pancreas of pig was used as enzyme material and the venom of Taiwanhabu was employed.

According to the figures in tables IX and X (pp. 192-193), it was observed that practically no perceptible activation was noticed upon the cleavage of either leucylglycine or alanylglycine in each case of using the original enzyme extract, residual solution and eludate.



Curves:

- I. When original enzyme extract, C_E 7.30,
 - II, When residual solution C_E 27.36,
 - III, When eludate, C_E —18.24,
- } were used

Relation between the activation power of the venom of Taiwanhabu and the time of contact of venom and enzyme.

In a similar way to the experimental process in a previous paper,² each enzyme-venom mixture was kept standing at 40° for varying lengths of time, and its activation power of each sample upon the LG-cleavage was tested. For the purpose of this experiment, the dried liver of tortoise was used as enzyme material and the venom of Taiwanhabu was employed.

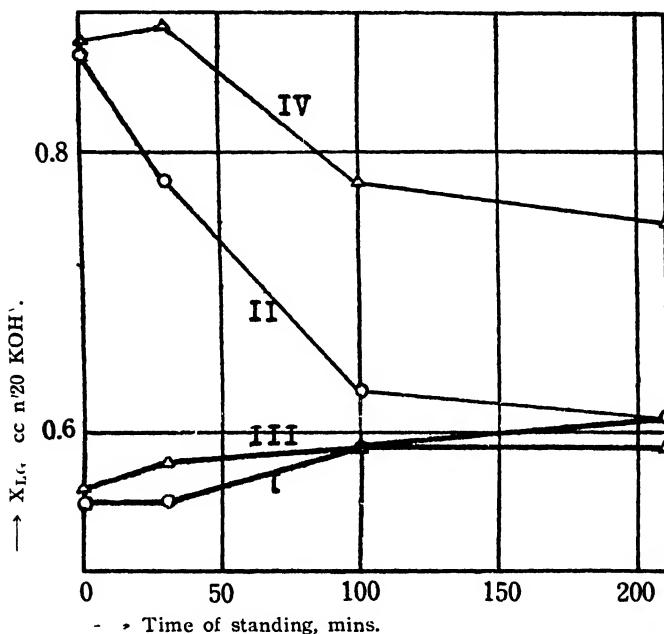
The results were shown in Fig. II (p. 186) and tables XII and XIII (p. 194).

According to these figures, it was observed that the activation was more stable in the eludate than in the original enzyme extract.

FIG. II.

Curves illustrating the activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of the original enzyme extract and eludate obtained from the dried liver of tortoise when the mixture of venom and enzyme was left standing for varying lengths of time at 40°.

The curves correspond to the figures in table XII.



Curves: I, When original extract, C_I 9.10, C_V -0
 II, When original extract, C_I 9.10, C_V 4.0
 III, When eludate, C_I 18.20, C_V 0
 IV, When eludate, C_F -18.20, C_V -4.0 } were used.

SUMMARY

As the enzyme material, dried liver of tortoise, dried kidney or pancreas of pig were used and each original enzyme extract was purified by the adsorption with aluminium hydroxide C_I and by the successive elution of the adsorbate with dilute ammonia solution and the activation power of the venom of Taiwanhabu upon the LG- and AG-cleavages was tested with each original enzyme extract, each re-

sidual solution and each eluate. The results thus obtained are summarized in the following :

- 1) Activation-relations similar to the case of each original enzyme extract were also found in the cases of each corresponding residual solution and eluate. (cf. table II, p. 188).
- 2) The degree of the activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of dried kidney of pig or dried liver of tortoise, increased in the case of using the eluate, while it decreased in the case of using the residual solution as compared with the case of using the corresponding original enzyme extract. (cf. table II, p. 188).

Therefore, according to the definition settled with regard to the purity of enzyme solution (see p. 183), it might be regarded that, in the present cases, the degree of the activation power named, increased as the purity of each enzyme solution increased. (The same tendency was also observed when similar test was made with the venom of Taiwankobura, using dried kidney of pig as enzyme material).

- 3) In a previous investigation,² it was observed that the degree of the activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of dried liver of tortoise decreased as the time of contact between the venom and the enzyme was lengthened.

In the present investigation, as clearly shown by tables XII, XIII (p. 194) and Fig. II (p. 186), it was found that the decreasing degree of the activation power as above observed, was far lessened in the case of using the eluate than in the case of using the corresponding original enzyme extract. Therefore, according to the definition above mentioned, it might be regarded that the decreasing degree of the activation power as above described was also far lessened as the purity of each enzyme solution increased.

TABLE II

Activation power of the venom of Taiwanhabu and Taiwankobura upon the LG- and AG-cleavages by dipeptidase of the original enzyme extracts, residual solutions and eludates obtained from various kinds of enzyme materials

The data correspond to the figures in tables IV, VII, X and XV

Kinds of enzyme solutions		Kinds of venoms		
		Taiwanhabu	Taiwankobura	
		AG	LG	LG
Dried kidney of pig	Original extract	—	+	+
	Residual solution	—	+	+
	Eludate	—	+	+
Dried liver of tortoise	Original extract		+	
	A Residual solution		+	
	Eludate	—	+	
	Original extract		+	
	B Residual solution		+	
	Eludate		+	
Dried pancreas of pig	Original extract	—	—	
	Residual solution	—	—	
	Eludate	—	—	

Note In this case, experiments were carried out in the main purpose of making the comparative test on the activation of each fraction of the enzyme extracts. Test with either various peptides or various snake venoms will be reported later. (—) cf. the results in the 4th report (Soon to be published as Mem. of the Fac. of Sci. and Agr. Taihoku Imp. Univ., Formosa, Japan, Vol. IX, No. 6 Part IV (1936)).

TABLE III.

Activation power of the venom of Taiwanhabu upon the LG- and AG-cleavages by dipeptidase of the original enzyme extract, residual solution and eludate obtained from the dried kidney of pig.

20 cc of enzyme extract I+5.4 cc of n/100 acetic acid+ 4.0 cc of Al (OH)₃ C_V+10.6 cc of 30% glycerine solution→40 cc 'pH 5.0 , centrifuged and filtered. To the residue were added 19.2 cc of n/100 ammonia solution and shaken well. After ca. 30 mins' standing, centrifuged and filtered. The residual solution and eludate thus obtained were immediately used without standing.

Kinds of enzyme solutions	C _I	X _{LG}				C _I	X _{AG}	
		C _V =0		C _V =4.0			C _V = 0	C _V =4.0
Original extract	6.84	0.40 0.40	0.40	0.75 0.73	0.74	1.82	0.45	0.53
Residual solution	27.36	0.35 0.34	0.35	0.50 0.50	0.50	7.30	0.31	0.37
Eludate	18.24	0.43 0.44	0.44	0.93 0.98	0.93	7.30	1.14	1.22

TABLE IV.

Calculation of the activation power of the venom of Taiwanhabu upon the LG- and AG-cleavages by dipeptidase of the original enzyme extract, residual solution and eludate obtained from the dried kidney of pig.

The data correspond to the figures in table III.

Kinds of enzyme solutions	Activation power			
	LG		AG	
	Per cent of activation A _{LG} ¹	Relative rate of activation ²	Per cent of activation A _{AG} ¹	Relative rate of activation ²
Original extract	+ 80	†	+ 4	—
Residual solution	+ 37	+	0	—
Eludate	+118	†	+ 2	—

Note: 1. A_{LG} and A_{AG} were calculated as noted on p. in 169 a previous paper.¹
 2. Relative rate of activation +, —, etc. as noted on p. 171 in a previous paper.¹

TABLE V.

Purity and yield of LG- and AG-splitting enzymes in the original enzyme extract, residual solution and eluate obtained from the dried kidney of pig.

Kinds of enzyme solutions	X calculated per $C_E=36.5$ ¹		Dried substance per $C_E=36.5$ ² mg.	X/dried substance		Comparison of purities ³		Yield ⁴ %	
	LG	AG		LG	AG	LG	AG	LG	AG
Original enzyme extract	2.13	9.02	4.7	0.453	1.919	1	1	100	100
Residual solution	0.47	1.55	3.8	0.119	0.404	0.26	0.21	22	17
Eluate	0.88	5.70	1.2	0.745	4.830	1.64	2.52	41	63

- Note: 1. X was calculated per $C_E=36.5$ from the data in table III, assuming that X is proportional to C_E (cf. p. 173 in a previous paper¹).
2. Dried substance was calculated per $C_E=36.5$ from the % determined.
3. Purity of the residual solution as well as that of eluate were compared with that of original enzyme extract, assuming that the purity of the latter is 1; for example, purity of LG-splitting enzyme in residual solution: $-0.119/0.453=0.26$.
4. Yield (%) of the enzyme of splitting each peptide was calculated under the same assumption as noted in case¹; for example, yield of LG-splitting enzyme in residual solution: $-0.47/2.13 \cdot 100=22$.

TABLE VI.

Activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of the original enzyme extract, residual solution and eluate obtained from the dried liver of tortoise.

20 cc of enzyme extract IIa or IIb + 8.0 cc of n/100 acetic acid + 4.0 cc of $Al(OH)_3$ + 8.0 cc of 30% glycerine solution \rightarrow 40 cc (pH 5.0), centrifuged and filtered. To the residue were added 19.2 cc of n/100 ammonia solution and shaken well. After ca. 30 mins' standing, centrifuged and filtered. The residual solution and eluate thus obtained were immediately used without standing.

(A) For enzyme extract IIa.

Kinds of enzyme solutions	C_E	X_{LG}	
		$C_V=0$	$C_V=4.0$
Original extract	9.10	0.58 0.57 0.56	1.06 1.06 1.06
Residual solution	18.20	0.35 0.35 0.35	0.51 0.51 0.51
Eluate	18.20	0.61 0.61 0.61	1.03 1.09 1.10

(B) For enzyme extract IIb.

Kinds of enzyme solutions	C_E	X_{LG}	
		$C_V=0$	$C_V=4.0$
Original extract	8.91	0.43 0.43 0.43	0.70 0.70 0.70
Residual solution	17.82	0.27 0.27 0.27	0.40 0.41 0.41
Eludate	17.82	0.28 0.28 0.27	0.60 0.61 0.61

TABLE VII.

Calculation of the activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of the original enzyme extract, residual solution and eludate obtained from the dried liver of tortoise.

The data correspond to the figures in table VI.

(A) For enzyme extract IIa.

Kinds of enzyme solutions	Activation power	
	Per cent of activation A_{LG}	Relative rate of activation
Original extract	+82	†
Residual solution	+40	+
Eludate	+75	†

Note: cf. foot note on table IV, p. 189.

(B) For enzyme extract IIb.

Kinds of enzyme solutions	Activation power	
	per cent of activation A_{LG}	Relative rate of activation
Original extract	+ 58	+
Residual solution	+ 31	+
Eludate	+111	††

Note: cf. foot note on table IV, p. 189.

TABLE VIII.

Purity and yield of LG-splitting enzyme in the original enzyme extract, residual solution and eluate obtained from the dried liver of tortoise.

(A) For enzyme extract IIa.

Kinds of enzyme solutions	$X_{I.G.}$ per $C_I = 36.4$	Dried substance per $C_I = 36.4$	$X_{I.G.}$ /dried substance	Comparison of purities	Yield %
Original extract	2.28	7.7	0.298	1	100
Residual solution	0.70	2.6	0.265	0.88	31
Eluate	1.22	2.5	0.480	1.61	54

Note: cf. foot note on table V, p. 190.

(B For enzyme extract IIb.

Kinds of enzyme solutions	$X_{I.G.}$ per $C_I = 35.64$	Dried substance per $C_I = 35.64$	$X_{I.G.}$ /dried substance	Comparison of purities	Yield %
Original extract	1.72	9.25	0.186	1	100
Residual solution	0.54	4.52	0.119	0.64	31
Eluate	0.56	2.16	0.259	1.39	33

Note: cf. foot note on table V, p. 190.

TABLE IX.

Activation power of the venom of Taiwanhabu upon the LG- and AG-cleavages by dipeptidase of the original enzyme extract, residual solution and eluate obtained from the dried pancreas of pig.

20 cc of enzyme extract III + 2.90 cc of n/10 acetic acid + 4.0 cc of Al (OH) C_I + 13.1 cc of 30% glycerine solution \rightarrow 40 cc (pH 5.0, centrifuged and filtered. To the residue were added 19.05 cc of n/100 ammonia solution 20 cc and shaken well. After ca. 30 mins' standing, centrifuged and filtered. The residual solution and eluate thus obtained were immediately used without standing.

Kinds of enzyme solutions	C_E	X_{LG}				C_I	X_{AG}	
		$C_V = 0$		$C_V = 4.0$			$C_V = 0$	$C_V = 4.0$
Original extract	15.66	0.55 0.54	0.55	0.56 0.57	0.57	31.32	0.39	0.46
Residual solution	31.32	1.02 1.02	1.02	1.00 1.02	1.01	62.64	0.49	0.57
Eluate	62.64	0.51 0.50	0.51	0.61' 0.61'	0.61*	93.96	0.11	0.18

*120 mins' digestion.

TABLE X.

Calculation of the activation power of the venom of Taiwanhabu upon the LG- and AG-cleavages by dipeptidase of the original enzyme extract, residual solution and eludate obtained from the dried pancreas of pig.

The data correspond to the figures in table IX.

Kinds of enzyme solutions	Activation power			
	LG		AG	
	Per cent of activation A_{LG}	Relative rate of activation	Per cent of activation A_{AG}	Relative rate of activation
Original extract	0	—	0	—
Residual solution	— 3	—	0	—
Eludate	+15	—	0	—

Note: cf. foot note on table IV, p. 189.

TABLE XI.

Purity and yield of LG- and AG-splitting enzymes in the original enzyme extract, residual solution and eludate obtained from the dried pancreas of pig.

Kinds of enzyme solutions	X per $C_E=62.64$		Dried substance per $C_R=62.64$	X/dried substance		Comparison of purities		Yield %	
	LG	AG		LG	AG	LG	AG	LG	AG
Original extract	2.20	0.78	26.0	0.0846	0.0300	1	1	100	100
Residual solution	2.04	0.49	25.2	0.0809	0.0194	0.96	0.65	93	63
Eludate	0.26	0.07	2.2	0.1192	0.0318	1.41	1.06	12	9

Note: cf. foot note on table V, p. 190.

TABLE XII.

The activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of the original enzyme extract and eluate of dried liver of tortoise when the mixture of venom and enzyme was left standing for varying lengths of time at 40'.

20 cc of enzyme extract IIb + 8.0 cc of n/100 acetic acid + 4.0 cc of Al (OH)₃ C₇ + 8.0 cc of 30% glycerine solution → 40 cc (pH 5.0), centrifuged and filtered. The filtrate was discarded. To the residue were added 19.2 cc of n/100 ammonia solution and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 20 cc of the eluate were added 10.8 cc of n/100 acetic acid and 9.2 cc of 30% glycerine solution (40 cc). The diluted eluate of pH 5.9 (= pH of the original extract) thus obtained was used. At the determination, C_R = 9.10 for the original extract and 18.20 for the eluate.

Time of standing, mins.	X _{LG}			
	Original extract		Eluate	
	C _V = 0	C _V = 4.0	C _V = 0	C _V = 4.0
0	0.55	0.87	0.56	0.88
30	0.55	0.78	0.58	0.89
100	0.59	0.63	0.59	0.78
210	0.61	0.61	0.59	0.75

TABLE XIII.

Calculation of the activation power of the venom of Taiwanhabu upon the LG-cleavage by dipeptidase of the original enzyme extract and eluate of dried liver of tortoise when the mixture of enzyme and venom was left standing for varying lengths of time at 40'.

The data correspond to the figures in table XII.

Time of standing, mins.	Activation power			
	Original extract		Eluate	
	Per cent of activation A _{LG}	Relative rate of activation	Per cent of activation A _{LG}	Relative rate of activation
0	+55	+	+54	+
30	+38	+	+50	+
100	0	-	+29	+
210	0	-	+24	+

Note: cf. foot note on table IV, p. 189.

TABLE XIV.

Activation power of the venom of *Taiwankobura* upon the LG-cleavage by dipeptidase of the original enzyme extract, residual solution and eluate obtained from the dried kidney of pig.

20 cc of enzyme extract I+5.4 cc of n/100 acetic acid+4.0 cc of Al (OH)₃, C_V+10.6 cc of 30% glycerine solution→40 cc (pH 5.0) centrifuged and filtered. To the residue were added 19.2 cc of n/100 ammonia solution (20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. The residual solution and eluate thus obtained were immediately used without standing.

Kinds of enzyme solutions	C _E	X _{LG}			
		C _V =0	C _V =2.0	C _V =4.0	C _V =6.0
Original extract	7.30	0.36	0.70	0.75	0.78
Residual solution	27.36	0.34	0.48	0.51	0.52
Eluate	18.24	0.39	0.74	0.84	0.92

TABLE XV.

Calculation of the activation power of the venom of *Taiwankobura* upon the LG-cleavage by dipeptidase of the original enzyme extract, residual solution and eluate obtained from the dried kidney of pig.

The data correspond to the figures in table XIV.

Kinds of enzyme solutions	Activation power					
	Per cent of activation A _{LG}			Relative rate of activation		
	C _V =2.0	C _V =4.0	C _V =6.0	C _V =2.0	C _V =4.0	C _V =6.0
Original extract	+94	+108	+117	†	††	††
Residual solution	+41	+ 50	+ 53	+	+	+
Eluate	+90	+115	+136	††	††	††

Note: cf. foot note on table IV, p. 189.

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THE POLAROGRAPHIC DETERMINATION OF NITRATES AND NITRITES

By

Matuo TOKUOKA

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INTRODUCTION

The determination of nitrates and nitrites has been a very important subject in analytical chemistry due to its great significance in agricultural and industrial chemistry. In common practice, the BUSCH,¹⁰⁾ the DEVARDA,¹¹⁾ the ARND,¹²⁾ the LUNGE¹³⁾ or colorimetric methods hitherto applied are, however, quite tedious. Another, simpler method should therefore prove to be of great service to the chemist.

Modern chemistry has a tendency to favour electrochemical determinations as the quickest and most accurate. Although electrochemistry is a rather young branch of chemistry, quite a number of studies concerning the reduction of these anions may be found already in the literature of the subject. They all are, however, complicated by the fact that only stable electrodes have been used; the results therefore, in spite of most careful manipulations, are often not satisfactory. If a determination is carried out, all of the nitrate resp. nitrite has to be reduced electrochemically; yet after that the final determination rests upon an ordinary titration of the reduction products.

Only the polarographic method of J. HEYROVSKÝ¹⁴⁾, employing a dropping mercury cathode, has shown any success in the solution of this problem. Various authors have used this method, but only for

theoretical purposes which will be described later on. He employs as cathode a dropping mercury electrode similar to that which B. KUČERA¹⁷⁾ used for the study of electrocapillary effects. The procedure is quite simple and the results are obtained quickly, as not all of the nitrate resp. nitrite, but only an infinitesimal part of it has to be electrolysed during the experiment. The deposition or reduction of any ions during electrolysis with the polarographic apparatus is shown by the current-increase on the current-voltage curve. If a galvanometer with a sensitivity of such an order as $10^{-8} \sim 10^{-7}$ Amp./mm/m. be used, even a concentration of 10^{-6} mols²¹⁾ pro litre of reduction-products upon the dropping mercury cathode can already be detected by the current-increase. Therefore, the sensitivity of the polarographic method for many substances may be compared with that of the spectroscopic method¹⁸⁾. Furthermore, the peculiar mechanism of the reduction of nitrates or nitrites enables us to determine a much smaller concentration of these anions by means of the polarographic method, as was already pointed out by the present author²⁶⁾.

As will be shown in the following pages, comparing electro-reduction by means of the stable electrodes with that on the dropping mercury cathode, the present author finds more suitability in the polarographic method for the determination of nitrates and nitrites.

The aim of the present work is to establish a polarographic method for the determination of nitrates and nitrites, setting forth in detail the necessary conditions for the practical application of this method. At the outset, in order to demonstrate the advantages of the polarographic method, the results obtained with stable electrodes were described and criticized.

The first part of the experiments consists in a study of the characteristics of the polarographic curves of nitrates and nitrites in neutral or alkaline solutions, including the cations of different valencies, which can reduce these anions on the dropping mercury cathode. The reduction of nitrites in acidic medium was also studied. From the results of these experiments the neutral solution of lanthanum

chloride was found to be the best indifferent electrolyte for the determination of nitrates and nitrites. Therefore, further studies were carried out exclusively with a neutral solution of lanthanum chloride. This solution gives a very positive reduction potential and the highest wave of nitrates and nitrites, which makes the measurement of the wave height very easy.

After the electro-reduction of nitrites and nitrates was theoretically considered, the determination of these anions and the separation of them from their mixture was studied. Furthermore, the influence of other anions, such as sulphate, phosphate, bromate, iodate etc upon the polarographic wave of nitrates and nitrites were also examined. In establishing the conditions for practical analysis with a polarograph, the determination of nitrates in fertilisers was carried out, the results of which compared with those obtained by the Devarda method, show a fair agreement with each other. From the results mentioned above, it may already be said, that this polarographic determination of nitrates and nitrites can be applied in practice.

I. THE ELECTRO-REDUCTION OF NITRATES AND NITRITES BY MEANS OF STABLE ELECTRODES

The first experiments concerning the electro-reduction of nitrates and nitrites were carried out by THORPE²⁾ and van der PLAATS³⁾ who employed platinum electrodes. The former expected to obtain hyponitrous acid (H_2, N_2, O_2) by electrolysing a potassium nitrate solution, but only obtained hydrogen at the cathode. Neither did the experiment of van der PLAATS, who reduced nitrites, produce this acid.

W. ZORN⁴⁾ employed a stable mercury cathode instead of a platinum electrode and found that nitrates and nitrites gave the same products during electrolysis. When nitrates were used, the electrolysis took about twice as long because the nitrates are first reduced to nitrites. On further reduction at the mercury cathode, hyponitrite is formed until no more nitrite remains in solution, after which also

ammonia and hydroxylamine are produced in larger amounts. S. TANATAR⁶⁾ obtained small amounts of hyponitrite by electrolysing sodium nitrite and barium acetate between platinum electrodes.

R. LUCKOW⁹⁾ and G. VORTMANN⁷⁾ again used platinum electrodes to electrolyse nitric acid. They found that concentrated nitric acid was reduced to nitrous acid. Dilute nitric acid could be reduced only in the presence of copper ions and sulphuric acid; the end-products were metallic copper and ammonium sulphate. Nitrates could similarly be reduced as long as no free alkali was present. On this basis it was possible to work out methods for the quantitative determination of nitric acid and nitrates, where the electrolytically formed ammonia was distilled off after addition of sodium hydroxide and then determined volumetrically. Details of the procedure are given by K. ULSCH⁸⁾ and L. H. INGHAM⁷⁾.

If we consider that during the reduction of nitrates or nitrites under varying conditions several end-products may be formed, such determinations become very problematical. Realizing this difficulty, R. IHLE¹⁰⁾ has studied in particular the current densities under which mainly ammonia is formed as a reduction-product of nitric acid. Using platinum electrodes, he found that more ammonia is formed at higher current densities, while below a certain current density nitric acid of the same concentration is no more reduced to ammonia. For instance with 17.85% HNO_3 , no ammonia is obtained at a current density of 0.0016 A/cm^2 , while with the same concentration at a current density of 0.0102 A/cm^2 , much ammonia is formed. He further found that the current density necessary to obtain just a trace of ammonia is:

0.0016 A/cm^2 for 14.67% HNO_3 ,

0.0112 A/cm^2 for 28.73% HNO_3 ,

0.0564 A/cm^2 for 43.34% HNO_3 ,

8.6000 A/cm^2 for 85.37% HNO_3 ,

From these figures it may be seen that the current density has to increase almost logarithmically with a linear increase of the con-

centration of nitric acid, in order to produce ammonia at the cathode.

As other end-products may also be formed besides ammonia during the electro-reduction of nitric acid, an investigation of the conditions, causing the preferential formation of one or the other, seemed necessary. J. TAFEL¹¹⁾ made a careful study of the relative amounts of hydroxylamine and ammonia, which he found as main products during the electrolysis of nitric acid in the presence of 50% sulphuric acid. This concentration of the sulphuric acid was chosen after experiment had shown that under these conditions a maximum amount of hydroxylamine was formed. Cathodes of the following materials were investigated: platinum, palladium, lead, cadmium, copper, silver, aluminium, tin, bismuth, nickel, carbon, and mercury; and further also amalgamated and tinplated electrodes. With mercury cathodes and well-amalgamated electrodes mostly hydroxylamine is formed. By proper arrangement, the formation of ammonia can thus be almost entirely suppressed and this method be used for a nearly quantitative transformation of the nitric acid into hydroxylamine salts. Copper electrodes, covered with spongy copper, on the other hand, practically reduce all the nitric acid to ammonia ($1\% \text{ NH}_4\text{OH}$). The other electrodes gave values lying between these two extremes. On the basis of these experiments, J. TAFEL could show that the methods of ULSCH and VORTMANN for the quantitative determination of nitric acid cannot be very accurate even when copper cathodes are being used.

The most important studies of the electro-reduction of nitrates and nitrites, i. e. of their reduction potentials and reduction-products have been carried out by E. MÜLLER and his collaborators¹²⁾. It was shown by plotting current-voltage curves that the influence of the cathode material upon the reduction of nitrates and nitrites, was in close connection with the observed difference of hydrogen overpotential at these metals. Special experiments proved that during the electrolysis of potassium nitrate on platinized platinum, 85% of the current was consumed for the formation of hydrogen. The corresponding

values are: for bright platinum 25%, for iron 2.5%*, for zinc 3.5%. No special experiment was made for mercury, but from one of the graphs it can be calculated that the results in this case would resemble those obtained with zinc. The reduction of sodium nitrite was also studied in detail and showed similar results. After determining the potentials at which nitrates, nitrites, hydroxylamine, and hydrazine were reduced on the various metals, E. MÜLLER (l. c.) came to the conclusion that during the electrolysis of nitric acid, neither hydroxylamine, nor hydrazine could exist, as they would be at once preferentially further reduced. He further stated that nitrites are reduced before nitrates on iron and zinc cathodes, while the reverse holds good for platinum electrodes. Thus only on platinum electrodes nitrates can be reduced to nitrites by keeping the cathode above a definite potential.

W. BÖTTGER¹⁷⁾ investigated the electro-analytical determination of nitrates. At the same time, he reviewed critically the work previously done on this subject. He considers the scheme



and segregates the determinations between those, where the ammonia is distilled off and then titrated, and those, where the diminution of a known quantity of acid due to the neutralisation by ammonia and hydroxyl ions is measured. The latter procedure has been impossible with the old methods where copper was deposited in a platinum vessel, because for the copper ions a corresponding number of hydroxyl ions was discharged at the anode. The most important source of error may be considered to be the formation of hydroxylamine at the cathode according to the scheme



BÖTTGER (l. c.), however, has found that this deficiency in the method is compensated mostly by other errors; e. g. the influence of copper,

* This value seems erroneous to the present writer, as, according to the graphs, iron should show practically the same results as platinum, viz. 25%.

which dissolves during the washing, and that of the indicator used could definitely be ascertained.

This period in which the electro-reduction of nitrates and nitrites was rather actively studied by many authors was followed by an interval of over twenty years in which no publications on this subject appeared. Finally in 1931 the problem was investigated again by L. SZEDELLEDY and B. M. SCHALL¹⁴⁾, who studied the electro-determination of nitrates. While in all previous methods sulphuric acid had been added to the solution, boric acid was employed by these authors, which enabled them to titrate directly the ammonia formed¹⁵⁾. To increase the conductivity, potassium sulphate (1 gr K_2SO_4 to 80-100 ccs electrolyte) was added to the electrolyte. The cathode was in one case platinum gauze, coated with copper; the anode consisted of a platinum spiral which was rotated quickly. In the other case, a nickel electrode was tried. It could be found that electrodes of pure nickel reduced only 30% of the nitrate present, while a nickel coated platinum electrode resulted in an 80% reduction. Finally by depositing the nickel under special conditions, quantitative reductions of the nitrate could be effected. As the following table I shows, the results are quite satisfactory.

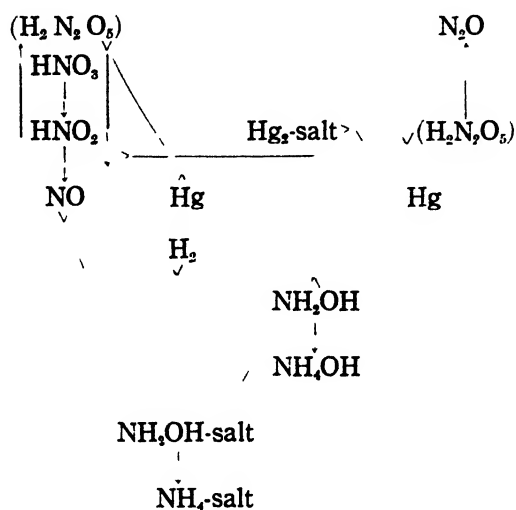
Table I.

Average results obtained with		
amount of salt used	Cu deposited on Pt	Ni deposited on Pt
0.1 g	+0.3 mg error	+0.3 mg error
0.2 g	-0.1 mg error	-0.2 mg error
0.3 g	-0.3 mg error	-0.6 mg error

Here too, the possibility exists that errors in one direction are compensated by others arising from the experimental conditions. This necessitates of course that the procedures outlined should be followed with painstaking accuracy.

Of further interest is the work of V. SIHVONEN¹⁶⁾ who studied the various steps in the reduction of nitric acid on stable mercury cathodes in 50% and 25% solutions of phosphoric acid or sulphuric

acid. With increasing potential and current density at the cathode, the following series of electrolytic reactions is obtained: HNO_3 , HNO_2 , NO , H_2 , hydroxylamine salts, and ammonia, resp. ammonium salts. The chief result is given in the following scheme, where the uncertain intermediate products are written in brackets. It is based on the employment of mercury cathodes.



In acid solution the compounds following below each other are formed at continuously rising cathodic polarisation. Nitrous acid and nitric oxide are formed without the evolution of hydrogen. If the current density is increased, reduction of the hydroxylamine, freed from the salt, is promoted.

In general, it may be concluded from the results with stable electrodes, that they are not suitable for the practical determination of nitrates and nitrites. Such analyses take too long due to the fact that the nitrates, resp. nitrites always have to be completely electrolysed out of the solution, which is a very difficult task even under the best conditions. That the end-products of the electrolysis always have to be determined at the end by an ordinary volumetric analysis is another reason why these methods have had no particular

advantage over the usual volumetric determination of nitrates and nitrites. As furthermore no detailed studies have been made concerning the influence of other ions which may be present in the solution to be analysed, the present author believes that none of these methods may be relied upon in analysing an unknown solution.

II. ELECTRO-REDUCTION OF NITRATES AND NITRITES ON THE DROPPING MERCURY CATHODE

Although numerous investigations have been already made with the polarograph in every branch of chemistry, the studies of the electro-reduction of nitrates and nitrites on the dropping mercury cathode are very few. Nitrite ions have been studied already by J. HEYROVSKÝ and V. NEJEDLÝ²¹⁾, but these authors found that this anion could not be reduced from neutral or alkaline solutions. A wave appeared on the polarographic curve only when the solution was made acid. Because however, the nitrites in acid solution decompose into nitric oxide and nitric acid, this is not a reduction of an anion, but, as the authors have determined, a secondary reduction of nitric oxide with atomic hydrogen. B. A. GOSMAN²²⁾ studied the influence of nitrates on the deposition of alkalies. He found that the nitrate ions cause a shift in potentials to more positive values, but he did not observe a reduction. J. RŮŽČKA²³⁾ observed that a polarographic wave of nitrates can be obtained, if in the electrolyte an excess of metallic ions, e. g. magnesium, calcium, strontium, barium or aluminium ions, is present. These results have been supplemented by the present author who studied the nitrate reduction in the presence of lanthanum, cerium, thorium, alkali metals and quarternary amino bases and their salts.

Analogously to what had been done with nitrates, the reduction of nitrites was also studied by the present author in the presence of various cations, especially in neutral and alkaline mediums.

The results of this investigation as well as those of other authors

have been brought together by the author and worked out into a method for the quantitative determination of nitrates and nitrites and for their separation.

The polarograph, which was used in this investigation, is of HEYROVSKÝ and SHIKATA system. The galvanometer (A) used in most parts of these experiments—unless otherwise specified—was that of d'Arsonval with a sensitivity of $7 \cdot 10^{-9}$ amp/mm/m with a half period of swing of 4 seconds, which is the best for these purposes. Sometimes another galvanometer was used with a sensitivity of $2 \cdot 10^{-8}$ amp/mm/m (Galvanometer B). Care was taken always to insure proper damping by introducing suitable resistances. In all the experiments described here, "dead damping" has been ascertained.

A. Nitrates

The concentration for a polarographic determination is usually 10^{-5} to 10^{-1} n. Such solutions have a rather large resistance, which has an influence upon the character of the reduction-wave and therefore it is desirable to work always with solutions of similar resistances. To make this possible, an indifferent electrolyte in a concentration of 10^{-1} to 10^{-2} n is usually added to those dilute solutions. Such indifferent electrolytes are those with cations, which have a very negative deposition potential, e. g. chlorides of ammonium, potassium, sodium, lithium and further those of the alkaline earths (calcium chloride and magnesium chloride are the best), and then tetramethyl ammonium chloride and tetraethyl ammonium chloride, these salts, being necessary if we wish to work in neutral solutions. For the electrolysis in alkaline mediums, the hydroxides of potassium, sodium, lithium or tetramethyl ammonium hydroxide resp. buffer solutions with pH of 7 are used. As acid medium, we employ 0.1 n resp. 0.01 n hydrochloric acid or buffer solutions. Before analysis always a blind test has to be performed with the pure indifferent electrolyte in order to determine the purity. After this test, the substance or solution to be investigated has to be added.

In the reduction of nitrates and nitrites these electrolytes have, however, a much deeper significance. Without an excess of certain electrolytes, no reduction of these anions takes place. Such electrolytes must contain any one of the following cations: $(\text{CH}_3)_4\text{N}^+$, $(\text{C}_2\text{H}_5)_4\text{N}^+$, Li^+ , Ca^{++} , Mg^{++} , Sr^{++} , Ba^{++} , Al^{+++} , La^{+++} , Ce^{+++} , Th^{++++} ; each of these has a specific influence which will be treated in a later chapter, but it may be mentioned here that the valency of these cations is of the greatest significance for the reduction-potential as well as for the general character and the height of the reduction wave.

a. *Electro-reduction of nitrates in quarternary amino bases and salts*

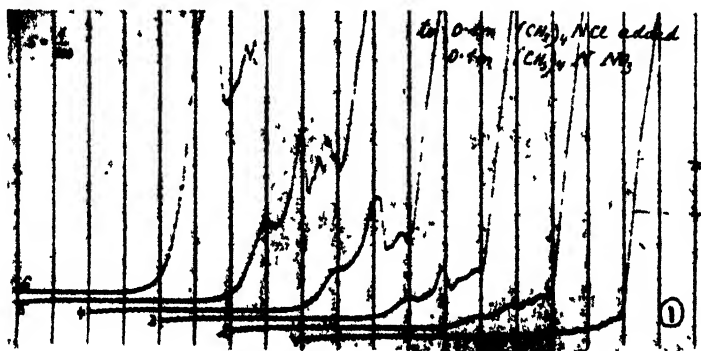
From a practical standpoint these experiments are of minor importance. But it is necessary also to study and investigate these problems for a complete treatment of the subject, and to make possible a theoretical explanation of the electrode processes.

Quarternary amino cations are reduced at the dropping mercury cathode only when the potential of the cathode has attained the value of 2.4 to 2.8 volts. This means that if we take a pure solution, e. g. a 0.1 m. solution of tetramethyl ammonium chloride, and electrolyze it, we get with increasing E. M. F. a residual current which reaches up to 2.4 V. We can therefore determine in this solution all components which have a more positive reduction potential.

Polarogram 1 may serve as an example. Curve 1 was obtained by electrolyzing 2.8 cc of 0.1 n. pure tetramethyl ammonium chloride. The electrolysis was started at 1.0 V; a 4 V. accumulator was used (abscissa=200 MV). It may be seen that at an applied E. M. F. of 2.8 V. the current intensity increases suddenly, attaining a very high value. In the further curves a 0.1 n solution of tetramethyl ammonium nitrate was added, in curve 2) 0.014 cc, 3) 0.028 cc, 4) 0.056 cc, 5) 0.112, 6) 0.224 cc. With increasing concentration of nitrate ions, a larger and larger wave appears on the curves,

which must be caused by an electro-reduction of nitrate ions, because the tetramethyl ammonium ion must be reduced at practically the same applied E. M. F., no matter if it is derived from the chloride

Polarogram 1.



or nitrate salt. The reduction of nitrate was further carried out in solution of tetraethyl ammonium chloride and in both iodides and bromides. The reduction potential of 2×10^{-3} n. nitrates from these solutions, compared to n. KCl is :

-2.26 v.	for 0.01 n.	tetramethyl ammonium chloride
-2.15 v.	0.1 n.	do.
-2.08 v.	0.5 n.	do.
-2.05 v.	1.0 n.	do.
-2.13 v.	0.1 n.	tetramethyl ammonium iodide
-2.08 v.	0.1 n.	tetraethyl ammonium chloride
-2.06 v.	0.1 n.	tetraethyl ammonium iodide
-2.15 v.	0.1 n.	tetramethyl ammonium hydroxide.

The reduction potentials of the nitrate ion from these solutions is very negative and exactly in the region where the alkali metals, resp. alkaline earths in traces are deposited at the mercury dropping cathode. These traces cause irregularities on the nitrate waves. Therefore also in these experiments tetramethyl ammonium nitrate free from alkali metals and earths were used, which were prepared by titration of purest tetramethyl ammonium hydroxide, kept in silver vessels, with dilute nitric acid.

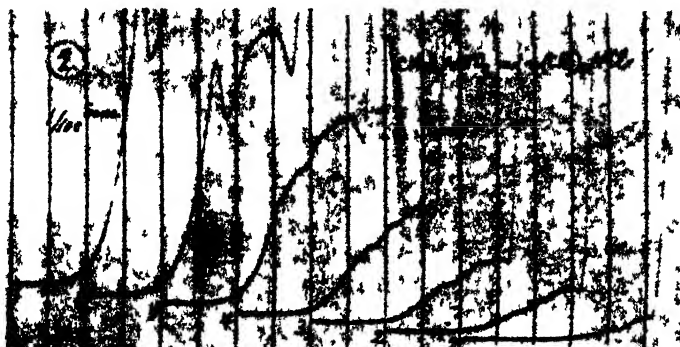
In cases where the polarographic waves are at such negative potentials, they are not quite so regular, especially in regard to the reduction of the tetramethyl ammonium ion. This is caused by the fact that the drop-time is a function of the potential to which the drop is charged and therefore in this part of the curve is much shortened and more or less irregular, all due to the influence of the much decreased surface tension of the mercury. At the deposition of tetramethyl ammonium chloride, this surface tension decreases almost to zero which is demonstrated by the fact that at such an applied E. M. F. the mercury instead of dropping, flows out from the capillary in a thin stream.²⁵⁾

On every wave due to the reduction of nitrate ions from solutions of salts or bases of quarternary amines, a maximum of the current intensity is formed which grows with increasing concentration of nitrate ions. Besides this generality the maximum depends also on what quarternary amine was used. It is higher in tetraethyl ammonium chloride than in tetramethyl ammonium chloride wherefore the size of the ion may be considered as the factor on which the height of the maximum depends. Polarograms 2, 3, and 4 show the course of reductions in n. tetramethyl ammonium chloride, in 0.1 n. tetraethyl ammonium iodide, and in 0.1 n. tetramethyl ammonium hydroxide respectively.

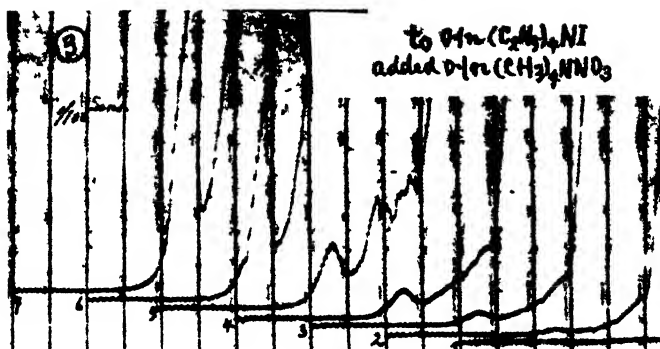
b. *Electro-reduction of nitrates in solutions of the alkali metals*

Potassium nitrate was already studied by B. A. GOSMAN²²⁾ who investigated the influence of anions upon the reduction of cations on the dropping mercury cathode. He could not observe a reduction of nitrates; he noticed however, that the presence of nitrates has an influence upon the deposition of potassium in so far as its reduction-potential is displaced to more positive values. The turning point of potassium from a normal solution of potassium chloride is much more negative than that from a normal solution of potassium nitrate as well as from more dilute solutions. (See Table I.)

Polarogram 2.



Polarogram 3



Polarogram 1

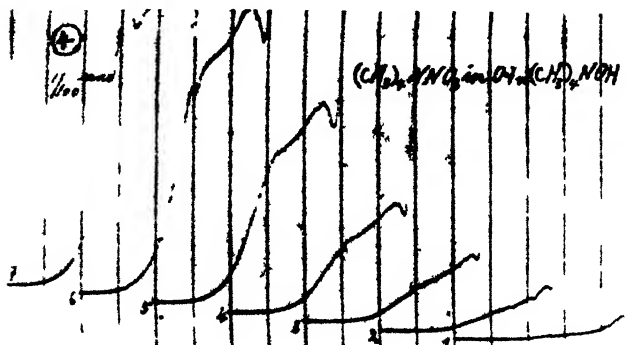


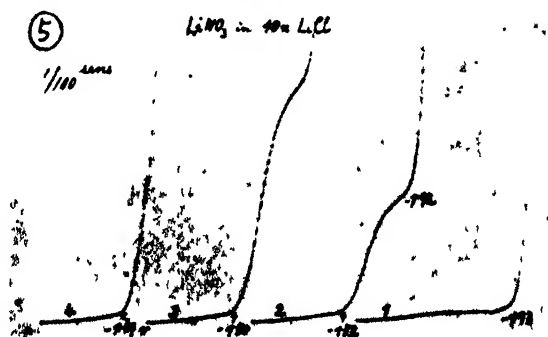
Table I.

Turning point of potassium

solution	from KCl	from KNO
1 n.	-1.88	-1.45
0.1 n.	-1.94	-1.60
0.01 n.	-2.00	-1.80

Polarographic curves of nitrates, instead of showing a sharp curve as in chloride solutions, show only a slowly increasing slope. The acidity or basicity caused a big displacement to the left or to the right respectively. GOSMAN came to the conclusion that here a secondary reduction of nitrates is caused by hydrogen atoms which are reduced at the cathode. The reduction then follows according to the equation $2H + NO_3^- \rightarrow H_2O + NO_2^-$. But in these investigations, GOSMAN never has obtained a polarographic wave wherefore it may not be said with certainty that here a reduction really takes place and also which is the reduction potential and the mechanism of the reaction. Only in the present special investigation of the nitrate determination, the author could succeed in finding the conditions under which a reduction of nitrates takes place and under which a wave is obtained on the polarographic curve. If such a wave is desired, the electrolyte must contain a large quantity of alkali cations, e.g. 10 n. lithium chloride. (polarogram 5). Curve 1 of this polarogram contains no nitrate and lithium is desposited at a potential of -1.93 volts. In the further curves lithium nitrate was added to give the following concentrations: curve 2) 0.001 n., curve 3) 0.02 n. and curve 4) 0.04 n. As may be seen, a wave appears on the polarographic

Polarogram 5.



contains no nitrate and lithium is desposited at a potential of -1.93 volts. In the further curves lithium nitrate was added to give the following concentrations: curve 2) 0.001 n., curve 3) 0.02 n. and curve 4) 0.04 n. As may be seen, a wave appears on the polarographic

curve which is proportional to the concentration of nitrate ions. Its turning point is in 0.001 n. nitrates -1.52 v. In table II are further given the potentials at which a 2×10^{-3} n. solution of nitrate is reduced from various lithium chloride solutions.

Table II.

2×10^{-3} n. LiNO_3 reduced from solutions of lithium chloride.

10^{-3} n. LiCl	π --2.122 v.	π (theoret.) 2.141 v.
2×10^{-2} „ „	-2.083	
10^{-1} „ „	-2.004	2.082
2×10^{-1} „ „	-1.982	
5×10^{-1} „ „	-1.967	
1 „ „	-1.891	2.023
2.5 „ „	-1.773	
5 „ „	-1.678	
10 „ „	-1.502	1.964

In the last column are given the deposition potentials of lithium from pure lithium chloride. From the table may be seen how the difference between the deposition potentials grows and therefore also how the possibility for a wave develops.

c. Electro-reduction of nitrates in solutions of alkaline earths

As for the above, the influence of bivalent cations on the reduction of nitrates was studied. Here not such a large excess of electrolyte is necessary as in the case of monovalent cations in order to obtain a wave of the nitrate reduction on the polarographic curve. The reduction potential of nitrates is here more than 400 MV more positive than in the presence of an equal concentration of alkali cations. In Table III are given the values of reduction potentials as they were found for various concentrations of nitrates in several solutions of the chlorides of the alkaline earths. All given values refer to the normal calomel electrode.

Table III.

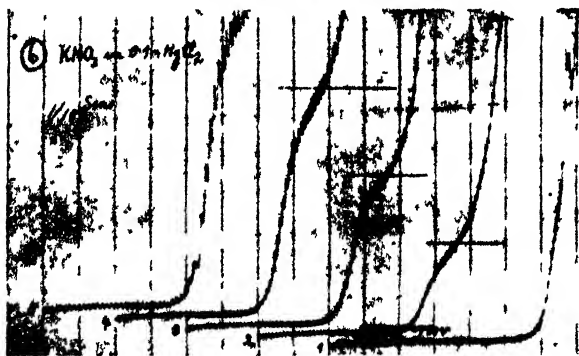
LiNO ₃ solution	in the presence of	τ
2×10^{-3} n.	0.1 n. MgCl ₂	-1.74 v.
2×10^{-3} n.	0.1 n. CaCl ₂	-1.78
2×10^{-3} n.	0.1 n. SrCl ₂	-1.79
5×10^{-3} n.	0.1 n. MgCl ₂	-1.71
5×10^{-3} n.	0.1 n. CaCl ₂	-1.76
1×10^{-2} n.	0.01n. MgCl ₂	-1.78
1×10^{-2} n.	0.1 n. MgCl ₂	-1.75
1×10^{-2} n.	1.0 n. MgCl ₂	-1.76

In spite of the fact that the reduction potentials of nitrates in the presence of these cations are almost the same, it can be seen that a solution of magnesium chloride serves best as an indifferent electrolyte because the polarographic wave in this case is best developed, i. e. it has the longest diffusion current with a smaller slope than the others. Thus the wave can be measured exactly and thereby a most accurate analysis of the nitrate concentration becomes possible. The barium ion is the worst because the reduction potential of barium is very positive and the wave does not develop properly, so that measurement of it becomes very difficult. For this reason, the measurements were mainly carried out in the presence of calcium and magnesium and only very few in the presence of strontium.

To illustrate this reduction, a few polarograms are here presented.

In polarogram 6, a solution of 0.1 n. potassium nitrate is added to 0.1n. magnesium chloride so that the concentration of nitrate was as follows: curve 1) pure MgCl₂, 2) 1.5×10^{-3} n. NO₃⁻, 3) 2.4×10^{-3} , 4)

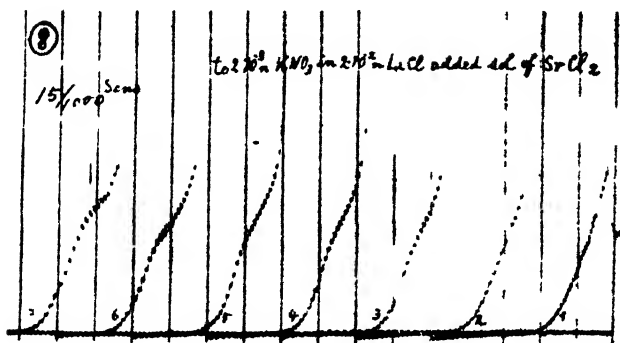
Polarogram 6.



Polarogram 7



Polarogram 8.

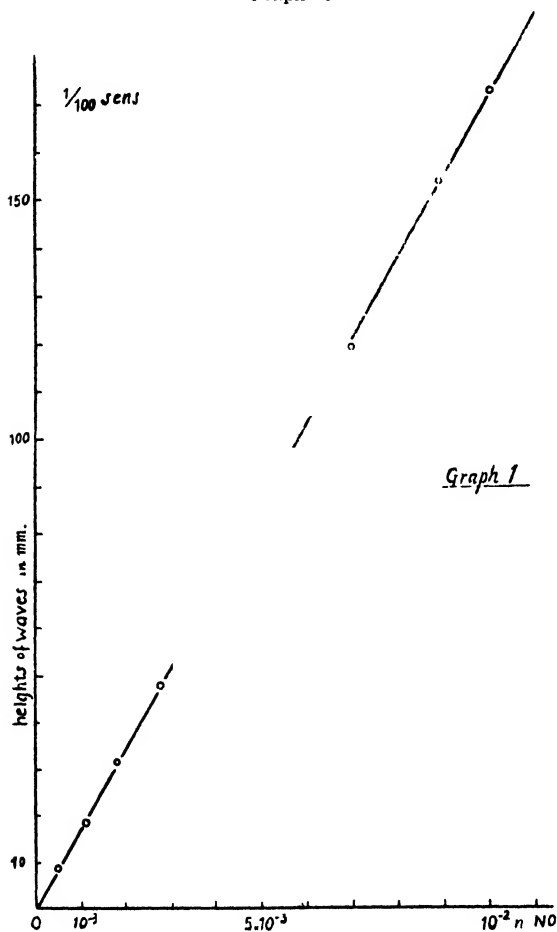


Polarogram 9



3.7×10^{-1} , and 5) $5 \times 10^{-1} n$. All curves start at -1.0 v.; a 4 volt accumulator was used. As may be seen, the diffusion currents are by no means very pronounced. We can therefore not use the usual method of measuring the height of a wave by drawing parallel lines

Graph 1.



over the top of the oscillations of the curve, but must draw those parallel lines in this case through the points of inflection of the curves. Polarogram 7 shows the reduction of nitrates in a solution of 0.1n. strontium chloride. Here a maximum appears with larger concentrations of the nitrate (as also in the presence of calcium) and the wave cannot be measured with accuracy.

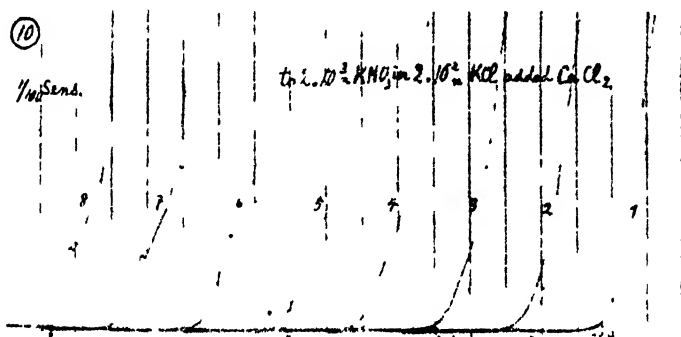
If waves obtained by the same concentration of nitrates in various concentrations of the indifferent electrolyte are compared, it will be seen that the kind (Mg, Ca, Sr, Ba) and concentration of the added indifferent electrolyte has no influence upon the height of the wave (see polarogram 8 for strontium and polarogram 9 for lithium). But the height of the wave increases proportionally

pared, it will be seen that the kind (Mg, Ca, Sr, Ba) and concentration of the added indifferent electrolyte has no influence upon the height of the wave (see polarogram 8 for strontium and polarogram 9 for lithium). But the height of the wave increases proportionally

with the concentration of nitrates. To 10 cc 0.1 n. pure magnesium chloride, solutions of 0.010 and 0.100 n. lithium nitrate were added; the corresponding actual concentrations were calculated and the heights of the waves obtained with 1/100 of the galvanometer sensitivity were measured. The results are plotted in graph 1 and show a straight line.

As in the case of the alkali metals, here also the concentration of the indifferent electrolyte has an influence upon the reduction potential, as may be seen from polarogram 10. To a solution containing

Polarogram 10.



2×10^{-3} n. potassium nitrate in 2×10^{-2} n. potassium chloride, (curve 1) was added calcium chloride till concentrations from 10^{-3} n. (curve 2 to 10^{-1} n. (curve 8) were obtained. For an easy comparison of the results in each subsequent curve, the original curve without calcium chloride was drawn in. With increasing concentration of calcium chloride the reduction potential of nitrates is shifted, but faster as in the case of lithium chloride, so that here not such a large concentration of the indifferent electrolyte is necessary for the formation of a wave.

If we electrolyze solutions where nitric acid is present, the current intensity increases already at -1.4 v. This current is due to the reduction of hydrogen ions on the dropping mercury cathode. If the concentration of nitric acid becomes too large, no wave from the hydrogen ion reduction can be obtained any more, the current increases too much and the light reflected from the galvanometer mirror goes off the photographic paper even when the sensitivity is very small. Naturally in such a case the nitrate wave, which comes later than the hydro-

Polarogram 11



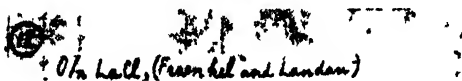
gen wave, cannot be obtained. Polarogram 11 shows curves obtained with very small concentrations of nitric acid. To 0.1 n strontium chloride (curve 1) 0.1 n. nitric acid has been added. Two waves are obtained (see curve 3) the first at about -1.4 v. is due to the reduction of hydrogen ions and the second one at about -1.8 v. is a nitrate wave. With larger concentrations (curve 5) on both waves maxima appear.

In basic solutions where it was possible (i. e. in saturated calcium hydroxide and 0.1 n. barium hydroxide) the reduction of nitrates is the same as in neutral solutions.

d. *Electro-reduction of nitrates in solutions of lanthanum chloride, cerium chloride, (aluminium chloride)*

It was seen that nitrates were reduced at a potential about 400 MV more positive from solutions of bivalent metals than from those of univalent metals. It was to be expected therefore that also trivalent metals would have an influence upon the electro-reduction of nitrates and that a further shift of potential would take place.

Polarogram 12.



As the chlorides of trivalent metals easily hydrolyze, the one with the least tendency to hydrolysis, lanthanum, was chosen first in order to avoid complications. For the investigation always lanthanum chloride (Merck puriss.) was used because other makes are much contaminated with impurities. Polarogram 12 gives an example of a 0.1 n. solution of lanthanum chloride prepared from a preparation of Fränkel and Landau (reinst), which was electrolyzed with the dropping mercury cathode.

It was found to contain

	in solution	by weight
Copper	6.6×10^{-6} n.	$2.4 \times 10^{-4}\%$ of the sample
Lead	$2. \times 10^{-6}$ n.	$1.2 \times 10^{-3}\%$
Zinc	$3. \times 10^{-6}$ n.	$1.2 \times 10^{-3}\%$
Nitrate	6.7×10^{-4} n.	$4.9 \times 10^{-3}\%$.

The Merck sample only showed a trace of nitrate in a concentration smaller than 1×10^{-7} n. in an 0.1 n. solution of lanthanum chloride, while all other metals were polarographically not detectable.

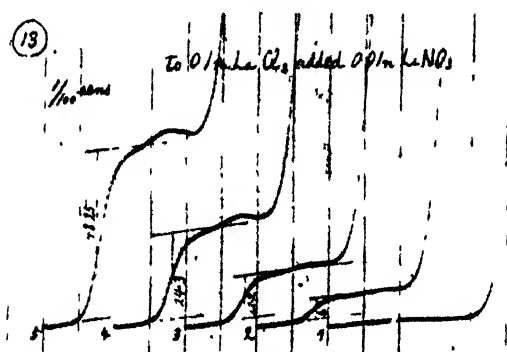
By electrolysis of a solution of pure 0.1 n. lanthanum chloride a polarographic curve is always obtained having a residual current up to -1.9 v., where the lanthanum is electro-reduced. Here the hydrogen exponent (due to hydrolysis) is $\text{pH} = 5.5$. At such concentrations no wave appears on the polarographic curves indicating the deposition of hydrogen ions, therefore cathodic phenomena may be investigated easily if they occur before the applied E. M. F. has reached -1.9 volt.

As in former experiments, a solution of pure lithium nitrate with exactly determined concentration was added to 10 cc 0.1 n. lanthanum chloride, so that the nitrate concentration was as follows: Polarogram 13 curve 1) zero, 2) 2.2×10^{-4} n., 3) 4.5×10^{-4} n., 4) 9.5×10^{-4} n., and 5) 1.85×10^{-3} n. With increasing concentration of nitrates a wave develops on these polarographic curves with a turning point between

1.2 and -1.3 v. due to the reduction of nitrate ions (all curves were started with an applied E. M. F. of 1.0 V.).

The diffusion current already present in small concentrations of nitrate has a marked and characteristic curvature which grows with an increasing concentration of nitrate ions and if it is large enough, begins to form a wave. This second increase of current is probably due to the electro-deposition of ammonia, which is the reduction product of the first stage of reduction of the nitrate anion, as has been pointed out before by the present author.²⁰⁾ If the height of these waves has to be measured, the parallel lines are drawn over the top of the oscillations of the first part of the wave (see curve 2, polarogram 13) when the con-

Polarogram 13.



centration is small; if it is greater, the parallel line is drawn through the inflection point of the diffusion current (curve 5, polarogram 13.)

Many polarograms were made for various concentrations of nitrate ions in the presence of 0.1 n. lanthanum chloride, the height of the waves measured and recalculated for a 1/100 sensitivity of the galvanometer. The results are shown in Table IV.

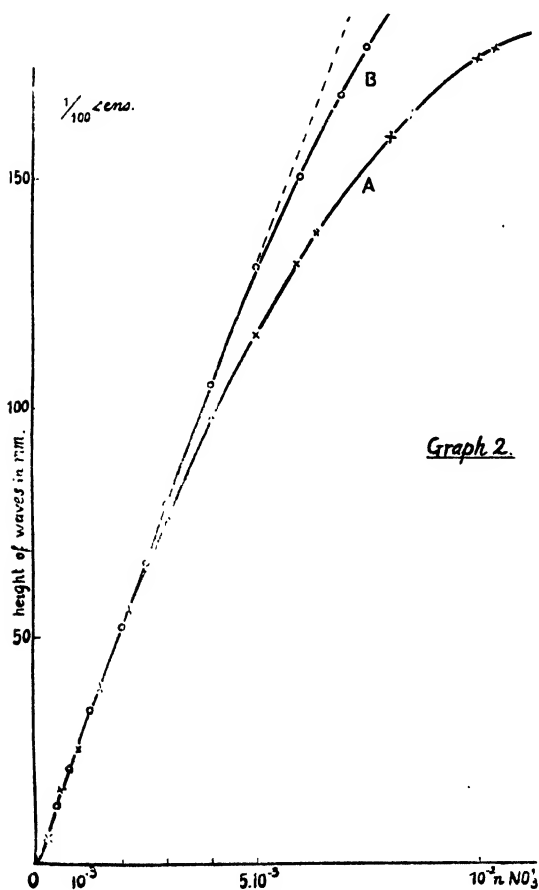
Table IV.

concentration of NO^-	height of wave
2.5×10^{-4} n.	6.5 mm
$5. \times 10^{-4}$ n.	13 mm
$8. \times 10^{-4}$ n.	21 mm
$1. \times 10^{-3}$ n.	26.5 mm
$2. \times 10^{-3}$ n.	52 mm
$3. \times 10^{-3}$ n.	77 mm
$4. \times 10^{-3}$ n.	98 mm
$5. \times 10^{-3}$ n.	116 mm

$6. \times 10^{-3}$ n.	133 mm
6.5×10^{-3} n.	140 mm
$8. \times 10^{-3}$ n.	159 mm
8.5×10^{-3} n.	164 mm
$1. \times 10^{-2}$ n.	176 mm

These values are plotted in graph 2 and thus curve A was obtained. From this it may be seen that the height of the wave grows

Graph 2.



Graph 2.

linearly with the nitrate concentration only up to 0.002 n. after which it already grows more slowly. In order to explain this peculiarity, the same experiments were carried out once more with 0.25n. lanthanum chloride instead of the 0.1 n. solution as above. The values obtained for the height of the wave at various nitrate concentrations, were also plotted on graph 2 (curve B). In this case the linear relation between nitrate concentration and the height of the wave goes as far as 0.005 n. One may conclude therefore, that the concentration of lanthanum chloride

here has an influence upon the height of the wave. This could be confirmed by the following experiments. The smallest concentration

of lanthanum ions was determined, which is sufficient to entirely reduce all of the nitrate ions present in the solution. To a solution with an exactly known nitrate concentration, a solution of lanthanum chloride, which contained also nitrate ions in the same concentrations, was added so that the nitrate concentration was constant during the experiment at all times. (Polarogram 14.)

Polarogram 14.

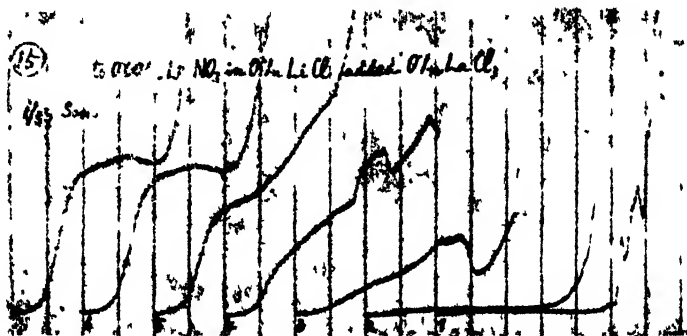


Due to the importance of this part of our investigations, it is worth while to give a more detailed description of polarogram 14. The electrolysis was carried out in the open air with a 1/100 sensitivity of the galvanometer and a 4 volt accumulator. All curves were started at -0.8 v. The original solution was 10 cc 0.1 n. tetramethyl ammonium chloride containing 2×10^{-3} n. tetramethyl ammonium nitrate (curve 1); then a mixture of 0.1 n. lanthanum chloride, 0.1 n. tetramethyl ammonium chloride, and 2×10^{-3} n. tetramethyl ammonium nitrate was added and the following concentrations of lanthanum obtained for the subsequent curves: (2) 10^{-3} n., (3) 5×10^{-3} n., (4) 10^{-2} n., (5) 2×10^{-2} n., (6) 5×10^{-2} n. and (7) 10^{-1} n. It may be seen that a concentration of 10^{-3} n. lanthanum ions is still insufficient to produce a nitrate wave. As soon, however, as the lanthanum ion concentration becomes 5×10^{-3} n. already an increase of current intensity at about 1 volt is observed, which continues up to the lanthanum deposition. With further additions of lanthanum ions, the continuous current increase changes into a wave

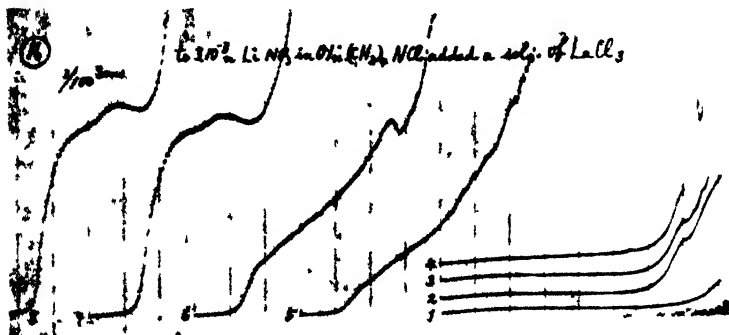
which finally reaches a limit when the concentration of lanthanum chloride becomes 10^{-1} n. Also in the case where the concentration of lanthanum ions is insufficient to produce a nitrate wave (as in curve 3), the continuous current increase reaches the same limiting value as that of the wave. This is due to the fact that with an increasing potential a greater amount of lanthanum ions is always adsorbed on the electrode thus causing there a greater concentration of lanthanum which is able to bring about the reduction of more and more nitrate ions. This effect will be treated further in the theoretical part of this paper.

If different concentrations of nitrates are used, also the amount of lanthanum ions necessary to produce the limiting nitrate wave changes in proportion. Polarograms 15 and 16 show such a relation.

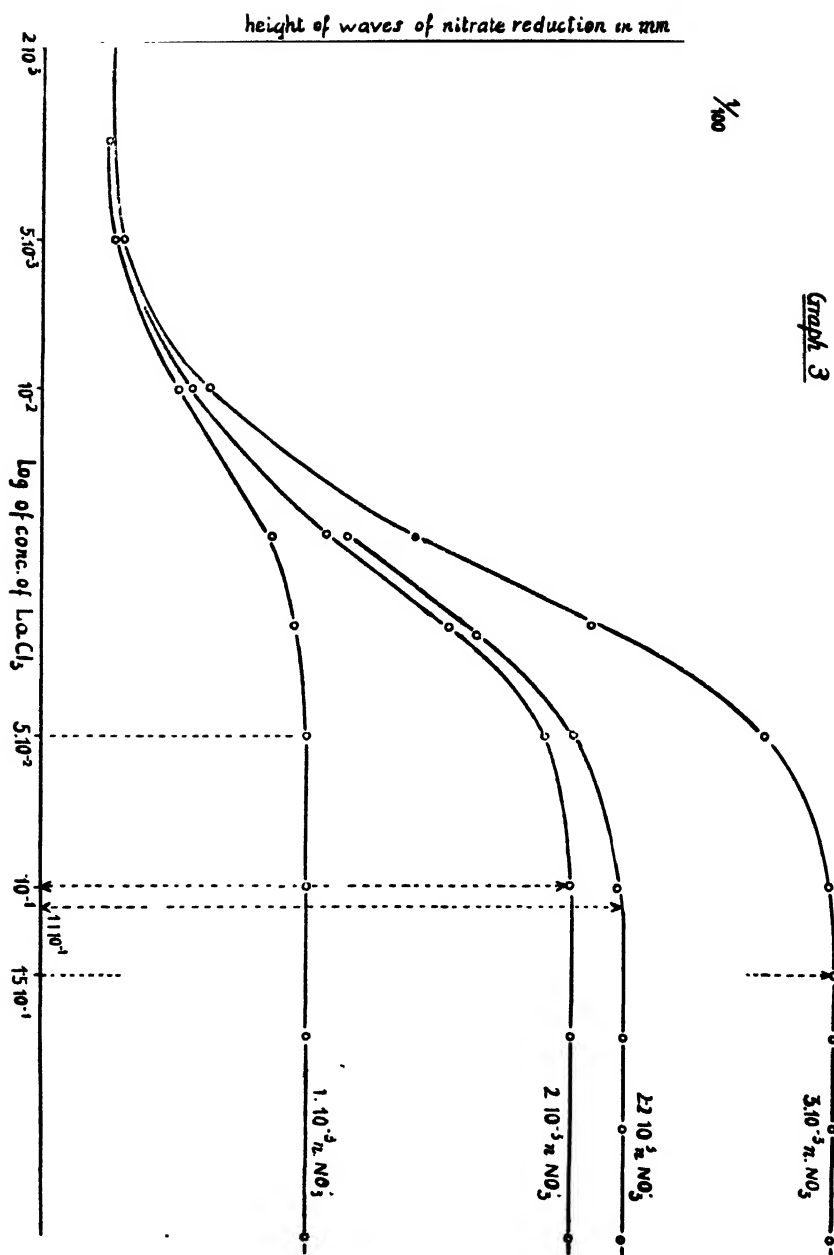
Polarogram 15



Polarogram 16



Graph 3.



The original solution is 20 cc of 0.1 n. lithium chloride, which contains 0.001 n. nitrate ion. To this solution is added 0.1 n. lanthanum chloride solution containing 0.001 n. nitrate ion in the following way (Polarogram 15):

Curve :	2	3	4	5	6	7
LaCl ₃ Sol. added :	0.2 cc	0.4	0.8	1.6	3.2	6.4

The original solution consists of 10 cc 0.1 n. tetramethyl ammonium chloride and 0.3 cc 0.1 n. lithium nitrate, to which is added 0.1 n. or 1 n. solution of lanthanum chloride. The concentration of lanthanum ion in the mixed solution is shown as follows (Polarogram 16):

Curve :	3	4	5	6	7	8
La-ion in n :	$5 \cdot 10^{-4}$	10^{-3}	$5 \cdot 10^{-3}$	10^{-2}	$5 \cdot 10^{-2}$	10^{-1}

This relation was tested in many electrolyses: some of the results obtained are plotted on graph 3. It can be seen that the height of the wave reaches a definite limit when the concentration of lanthanum ion is about 50 times that of the nitrate ions. Table V gives a summary of the results.

Table V.

Concent. of nitrate ion in n.	Concent. of La ⁺⁺⁺ ion necessary for maximum height of nitrate wave in n.	Conc of La ⁺⁺⁺ ion — Conc. of NO ₃ ⁻ ion
1 $\times 10^{-3}$	5 $\times 10^{-2}$	50
2 $\times 10^{-3}$	9.3×10^{-2}	47
2.2 $\times 10^{-3}$	1.1×10^{-1}	50
2.15×10^{-3}	1.1×10^{-1}	51
3 $\times 10^{-3}$	1.5×10^{-1}	50
5 $\times 10^{-3}$	2.7×10^{-1}	54

The concentration of lanthanum ions has an influence not only upon the height of the nitrate wave, but also upon the reduction potential of nitrates, which of course is dependent mainly on the concentration of nitrate ions. This influence can be seen from the several curves on polarogram 14, from which the relation between

the concentration of La^{+++} ion and the reduction-potential was obtained; the latter is measured against a normal calomel electrode.

Curve:	2	3	4	5	6	7
$[\text{La}^{+++}]$ in n.	10^{-3}	$5 \cdot 10^{-3}$	10^{-2}	$2 \cdot 10^{-2}$	$5 \cdot 10^{-2}$	10^{-1}
π (v.)	-1.92	-1.06	-1.07	-1.09	-1.10	-1.12

The increase of the concentration of La^{+++} ion shifted the reduction potentials of nitrates to the more positive side. Furthermore, the reduction potentials, measured against a normal calomel electrode, for various nitrate concentrations are given in Table VI.

Table VI.

conc. NO_3^-	conc. LaCl_3	red. potl. NO_3^-	average
0.005 n.	0.1 n.	-1.23 v.	
0.005 n.	0.2 n.	-1.25 v.	
0.002 n.	0.1 n.	-1.22 ₆ v.	-1.22;
0.002 n.	0.1 n.	-1.23 ₀ v.	
0.002 n.	0.2 n.	-1.24 ₂ v.	
0.001 n.	0.1 n.	-1.28 v.	
0.001 n.	0.1 n.	-1.29 v.	-1.28 ₈
0.001 n.	0.1 n.	-1.27 ₆ v.	
0.001 n.	0.1 n.	-1.29 v.	

The presence of trivalent cations thus shifted the potential to more positive values by about 0.9 volt as compared to that of univalent cations, and by about 0.5 volt as compared to that of bivalent ions. This displacement of the reduction-potential may be seen clearly in polarogram 17, where to a solution containing 4.7×10^{-3} n. nitrate in 0.1 n. magnesium chloride (curve 1) an normal solution of lanthanum chloride was added, while the concentration of nitrate ions was kept constant (curves 3 and 4). The concentration of lanthanum chloride in curve 2 is 0.002 n., in curve (3) 0.01 n. and in (4) 0.3 n. While in curve 2 the course of the curve is but little changed, in curve 3 a new wave already appears at a potential -1.2 v. which also is due to a nitrate reduction. We therefore have

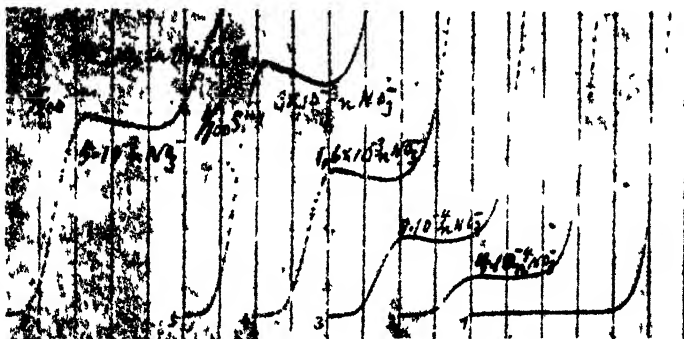
Polarogram 17



two nitrate waves in one curve, the first one caused by the lanthanum ions and the second by magnesium ions as the original wave in the curve 1. Now if still more lanthanum ions are added the first wave predominates over the second wave; the latter disappears at last, and the total reduction of nitrate is caused by the lanthanum ions, the wave being the same as when only lanthanum ions are present. These results are a clear proof of the displacement of the reduction-potential of nitrates.

The fact, that the limiting current reached in lanthanum or cerium solutions is larger than that obtained when the same concentra-

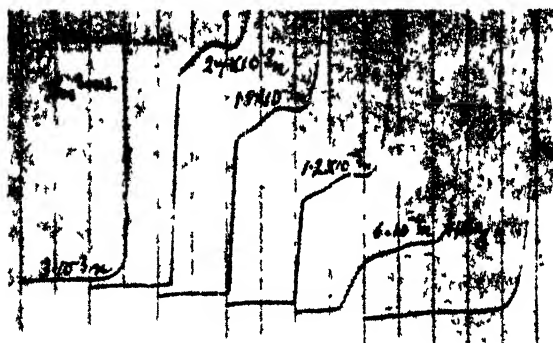
Polarogram 18



tion of nitrate is electrolysed in the presence of magnesium or other alkaline earth salts, was already recognized by a simple comparison of the wave height of nitrates in graph 1 (magnesium chloride) and

graph 2 (lanthanum chloride), which shows, that the wave height in the presence of a large excess of lanthanum chloride is about 50% higher than in the presence of mag-

Polarogram 19



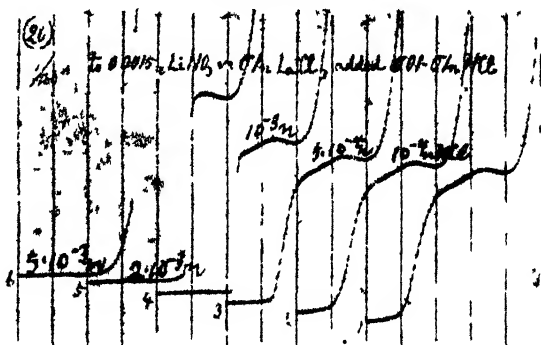
nesium chloride. This relation is also valid in general for other divalent ions and trivalent ions.

The reduction of nitrates in cerium chloride solutions proceeds in the same

way as in lanthanum chloride solutions. The reduction-potentials (polarogram 18) and the height of the wave are the same in both solutions, only the diffusion current in cerium chloride has a different specific character. An initial high current intensity is always observed in these waves, which at higher concentrations of nitrate ions becomes so large that the measurement of the wave height becomes very difficult.

The nitrate reduction in solutions of aluminium chloride was also investigated, but here due to the strong tendency of this salt to hydrolyze, the formed free acid had the same influence as will be described later. We have always obtained from aluminium chloride solutions a reduction-wave which is shifted towards the deposition-potential of hydrogen ions and starts with a discontinuity. The reduction-potential of aluminium

Polarogram 20



itself is much more positive than that of lanthanum, wherefore no

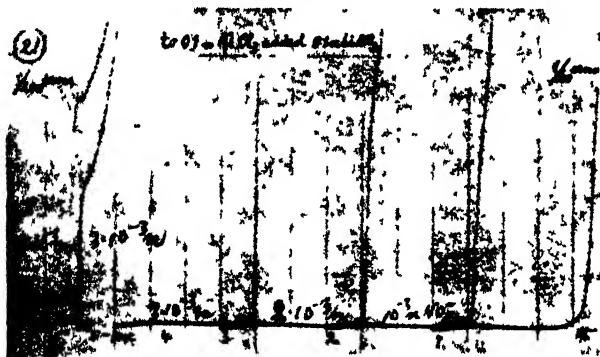
wave appears if the concentration of nitrate anions is small. This can be seen from polarogram 21. Only when the nitrate concentration has reached the order $10^{-1}n$, a wave with a small diffusion current appears.

The reduction of nitrates in the presence of thorium tetrachloride will be treated together with the reduction of nitrites.

The above experiments with lanthanum chloride were carried out in almost neutral solutions of this salt. Here, the influence of acid, oxygen and alkali metals upon the nitrate wave in lanthanum solutions was also investigated.

When acid is added to lanthanum solutions containing nitrates neither the nitrate wave nor that due to the deposition of hydrogen

Polarogram 21



ions appear at -1.2 v., but only one limiting current suddenly sets in at -1.4 v., which henceforth remains constant up to the deposition of lanthanum. (polarograms 19 and 20). Thus only one wave is observable on the curve, which must be due to the simultaneous reduction of both the hydrogen ions and the nitrate anions. The height of this wave is, however, considerably smaller than the sum of the limiting currents due to the reduction of the hydrogen ions alone and that of the nitrate ions, before the addition of the acid.

The addition of a small amount of alkali removes the discontinuous increase of the current at -1.4 v and introduces the ordinary bend on the current-voltage curves.

It is noteworthy that the admission of oxygen into the solution, e.g. when the solution is electrolysed in the open air, also alters the discontinuous step on the curve to an ordinary bend. Undoubtedly, when working in hydrogen atmosphere, the discontinuous increase of the nitrate reduction current is caused by the acidity of the lanthanum chloride solution; which undergoes hydrolysis. If, however, oxygen is present in the solution, hydroxyl ions are sent into the solution²⁴⁾ in the electro-reduction of oxygen, which makes the surface of the cathode alkaline; thus the alkalinity effects, shown by the continuous bend of the nitrate wave come in. As the discontinuous step is much more conspicuous and better measurable, it is advisable to avoid oxygen in solutions to be investigated polarographically for the presence of nitrate, especially when the nitrate concentration is very small.

From the foregoing it may be seen that fundamentally all trivalent cations have the same influence upon the reduction of nitrate ions. They give more positive reduction potentials and higher waves of nitrates for the same concentration of this anion than mono- or divalent cations. The tetravalent cation, i.e. thorium ion, can not be used for our purpose because of its unsuitable properties, as described in the next section. Therefore, the best indifferent electrolyte for the polarographic study of nitrates can be chosen among the trivalent cations. Of the trivalent cations, which have been investigated already, aluminium chloride has too strong a tendency to hydrolyse and cerium chloride shows a maximum phenomenon at the beginning of the nitrate wave, whereas lanthanum chloride has no such deficiencies at all. Furthermore it has a very suitable hydrogen ion concentration — 0.1 n lanthanum chloride solution has a pH of 5.5 at room temperature, the most suitable for analytical purposes —, and it gives a sharp wave of nitrate, the height of which is properly measurable.

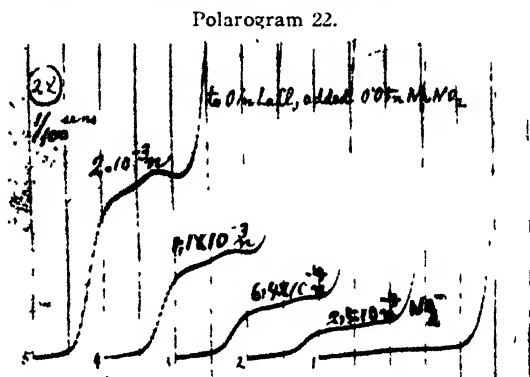
According to what has been said above, lanthanum chloride is selected as the best indifferent electrolyte for the polarographic study of nitrate-reduction, so that it has been used almost exclusively in analytical work as well as in further studies on this subject.

B. Nitrites

a. *Electro-reduction of nitrites in neutral and alkaline solutions*

Only in the case, where we use a neutral or alkaline solution, we can speak of a reduction of nitrites on the dropping mercury cathode. In these solutions nitrites are stable and no decomposition takes place as in an acid medium. Soon after some experience had been gained in nitrate reduction, it was found by the present author that nitrites are also reducible on the dropping mercury cathode. It could be determined that nitrites are reduced under the same circumstances and conditions as nitrates and that the reduction-potentials of these anions in neutral and alkaline medium are exactly the same, which makes it impossible to separate them in such solutions. Since the course and character of the reduction-waves are also the same, we have to assume that the cause and mechanism of the reduction are the same.

Polarogram 22 demonstrates some of the waves due to the reduction of nitrite ions from a lanthanum chloride solution. Like in the case of nitrate solutions, here also lanthanum chloride has



been found best for the reduction of nitrites. The heights of the waves from various concentrations of nitrite ions in 0.1 n. lanthanum chloride solution were measured and recalculated for a galvanometer sensitivity of 1/100. The results are given in Table VII.

Table VII.

concentr. of NO_2^-	height of the wave
$4. \times 10^{-4} \text{ n.}$	8.5 mm
$6. \times 10^{-4} \text{ n.}$	13 mm
$1. \times 10^{-3} \text{ n.}$	22 mm

1.5×10^{-3} n.	32.5 mm
$2. \times 10^{-3}$ n.	43 mm
2.5×10^{-3} n.	53 mm
$3. \times 10^{-3}$ n.	62.7 mm
$4. \times 10^{-3}$ n.	80.5 mm
$4. \times 10^{-3}$ n.	81 mm
4.8×10^{-3} n.	92 mm
$6. \times 10^{-3}$ n.	109 mm
7.1×10^{-3} n.	121 mm
$8. \times 10^{-3}$ n.	130.5 mm
8.9×10^{-3} n.	137 mm
$1. \times 10^{-2}$ n.	144 mm

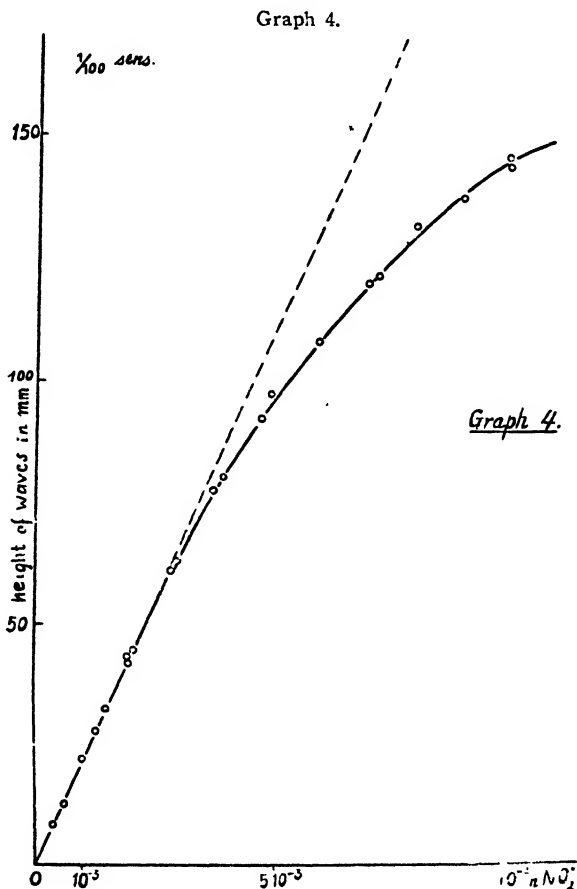
These values have been plotted in graph 4 where the concentrations of nitrite ions on the X-axis and the heights of the waves on the Y-axis are given. Thus a curve has been obtained which is very similar to curve A of graph 2. If we compare further the height of the waves due to nitrites and nitrates reduced from 0.1 n. lanthanum chloride solution with same sensitivity of the galvanometer, we obtain a constant ratio as is shown in Table VIII.

Table VIII.

concentration of NO_3^- or NO_2^-	height of the wave:		ratio of height of the waves $\text{NO}_3^-/\text{NO}_2^-$
	of NO_3^-	or NO_2^-	
4×10^{-3}	11	8.5	1.30
5×10^{-3}	13.5	10.5	1.29
10×10^{-3}	26.5	21.7	1.24
15×10^{-3}	40	32.5	1.26
20×10^{-3}	53	42.0	1.26
30×10^{-3}	77	62.5	1.25
	average		1.27

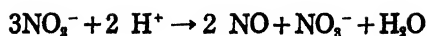
As may be seen, the average value of this ratio is 1.27; the significance of this value will be treated in the theoretical part of this paper.

Here too, as in the case of nitrates--where a 50 times larger concentration of lanthanum ions was found necessary for complete reduction--the concentration of lanthanum is of importance; however, even a smaller concentration will bring about the maximal height of the wave. Experiments, similar to those carried out with nitrates, showed that for a complete reduction of nitrites a 38-40 times larger concentration of lanthanum ions is necessary. In agreement with this it is found that the linear part of the curve on graph 4 is longer than that of nitrates. It reaches up to 0.0025 n. nitrite concentration for 0.1 n. lanthanum chloride solutions; this means that the ratio of these concentrations is $0.1 : 0.0025 = 40$, the same as determined in the experiments just mentioned.

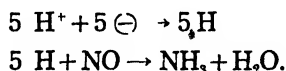


b. *Electro-reduction of nitrites in acid solutions.*

In an acid medium, the nitrite ion is unstable because the molecules of nitrous acid decompose according to the following equation:



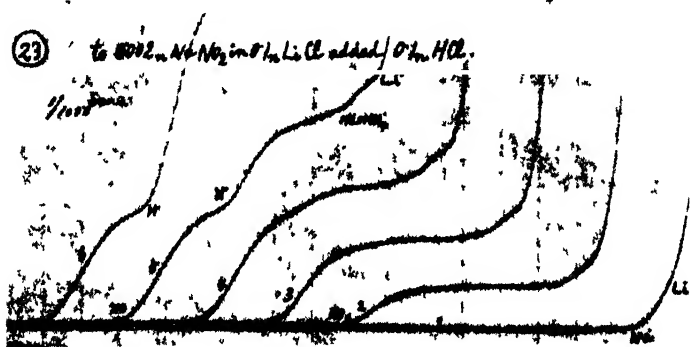
This removes the nitrite ions from the solution and leaves in its place nitric oxide and nitrate ions so that no reduction of nitrite ions on the cathode is possible. Acid solutions of nitrites were studied by J. HEYROVSKÝ and NEJEDLÝ²¹⁾ who determined that nitric oxide from 0.1 n. hydrochloric acid is reduced at -0.77 v. from the normal calomel electrode, and that the reduction proceeds as far as ammonia. This is considered as a secondary reaction with hydrogen atoms which have been formed by the reduction of hydrogen atoms which have been formed by the reduction of hydrogen ions.



According to this equation, 5 faradays are necessary for the reduction of one mol nitric oxide.

As hydrogen ions are necessary for the decomposition of nitrite ions to nitric oxide, as well as for the secondary reduction to ammonia, we always need an excess of acid for complete reduction. This is demonstrated in polarogram 23, where curve 1 was obtained

Polarogram 23.



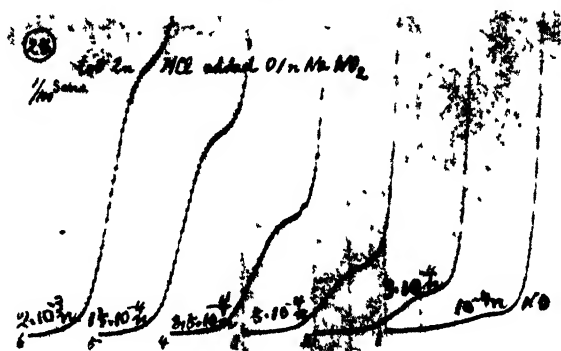
by electrolyzing 30 cc of neutral 0.002 n. sodium nitrite solution in 0.1 n. lithium chloride, which was freed from oxygen by a stream of hydrogen. The galvanometer sensitivity was 1/1000, a 3.8 volt accumulator was used, and the various curves were started at 0.0

volt. Curve 1 shows a residual current which goes to about 2 volt, where sodium and later on lithium are deposited. To this solution, 0.1 n. hydrochloric acid free from oxygen was added in such amounts that the following concentration of hydrogen ions were obtained: Curve (2) 0.0016 n., (3) 0.003 n., (4) 0.005 n., (5) 0.0065 n. and (6) 0.013 n. With an increasing concentration of hydrogen ions, a wave appears at -1.0 volt, which is due to the reduction of nitric oxide. If we have a hydrogen concentration equal to 0.0065 n. (curve 5), the first wave (due to nitric oxide) reaches a limiting height and another wave appears at about -1.4 v. which is due to the reduction of hydrogen ions, in addition a small, though gradually increasing, third wave, which is due to the reduction of sodium ions from the sodium nitrite and also of the formed ammonia.

Polarogram 24 is the reverse of polarogram 23; here to 10 cc of 2.0 n. hydrochloric acid an 0.1 n. solution of sodium nitrite was added. It can be seen that the nitrite wave, as long as it is small, is easily measurable and that

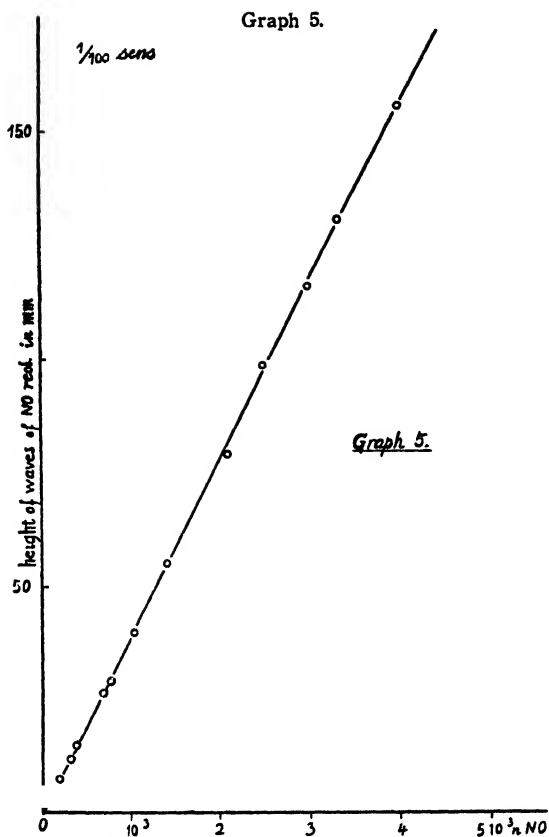
it grows with the increasing concentration of nitrite ions. To make a comparison with graphs 2 and 4 possible, graph 5 has been drawn, showing the relationship between the height of the wave

Polarogram 24.



due to the reduction of nitric oxide and the concentration of nitric oxide in 0.1 n. hydrochloric acid at a 1/100 sensitivity of the galvanometer. The concentration of nitric oxide was calculated in accordance with the above given equation as $2/3$ of the concentration of nitrite ions.

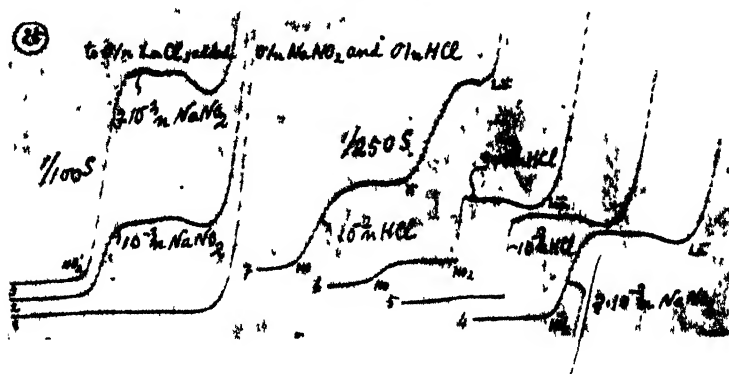
Instead of using free acids for the reduction of nitrites, the writer also used buffer solutions. For a complete reduction of 0.1 n. nitric oxide a buffer solution with a pH of 4 was here found sufficient.



This influence of the hydrogen ions on the reduction has been studied more in detail. A difference between the reductions of nitrates and nitrites should be found, as the nitrite ions decompose in the presence of hydrogen ions. Polarogram 25 may serve to demonstrate this. To 10 cc 0.1 n. lanthanum chloride (curve 1) 0.1 n. sodium nitrite was added, first 0.1 cc (curve 2), then 0.2 cc more (curve 3). This last curve was again repeated (curve 4) with a smaller sensitivity with which all the following curves were also taken.

Then 0.1 cc of 0.1 n. hydrochloric acid was added and the same discontinuity

Polarogram 25.



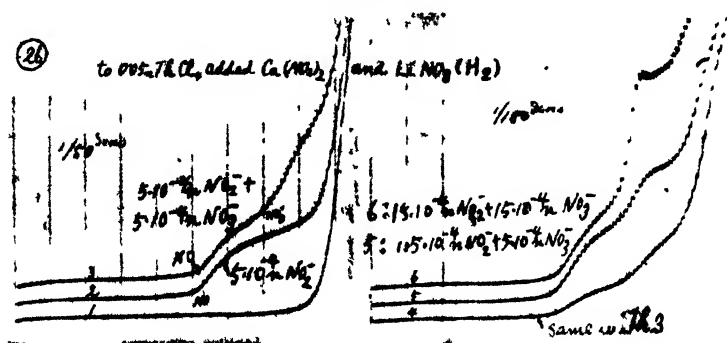
appeared which we have also observed in the case of nitrates. When furthermore 0.2 cc hydrochloric acid was added, a wave could be seen already at -1.0 v. which increased with further additions of hydrogen ions (curve 7); this wave is due to the reduction of nitric oxide.

c. *Electro-reduction of nitrite and nitrate in the solution of thorium tetrachloride.*

The solution of thorium tetrachloride has a double property. On the one hand thorium ion deposits at a more negative potential than the reduction-potential of nitrate ion, giving the wave of nitrate as shown in the polarogram; on the other hand, this solution has a strong tendency to hydrolyse. As a 0.1 n. solution is sufficient to decompose nitrite ion to nitric oxide, the reduction of nitrite in this solution is of the same nature with that in HCl solution. The reduction of nitrite occurs at a potential more positive than that of nitrate ion. The mixture of nitrate and nitrite show two waves on the polarographic curve, reduced in thorium tetrachloride solution, but the boundary between the two waves is not distinct, making the exact measurement of each wave impossible.

An example is shown in polarogram 26. A polarographic curve, which was obtained by electrolysing 20 cc of 0.5 n thorium tetrachloride can be seen in curve 1. To this solution were added in alternating succession 0.1 n calcium nitrite and 0.1 n lithium nitrate.

Polarogram 26.



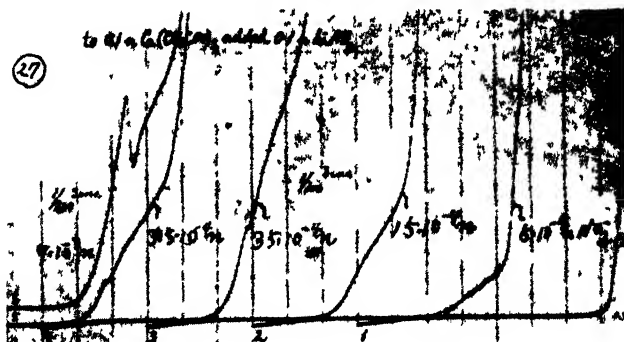
We see from the polarographic curves that here the reduction-potentials of these anions are different. Two waves are formed, the first of which appears after the addition of nitrites. the second one after nitrates had been added. However, the apparent reduction of nitrites is really a reduction of nitric oxide as could be determined by a detailed study.

In the extensive study of these curves it was found that this sort of reduction is not satisfactory for a quantitative determination of both components. Both waves can only be measured with difficulty, furthermore their height changes with an increase in concentration of one or the other component. For that reason thorium solutions can only be used for qualitative analysis and only then, if both components have not very greatly differing concentrations. An excess of nitrate ions makes even this qualitative test impossible.

III. THE INFLUENCE OF ANIONS ON THE ELECTRO-REDUCTION OF NITRATES AND NITRITES

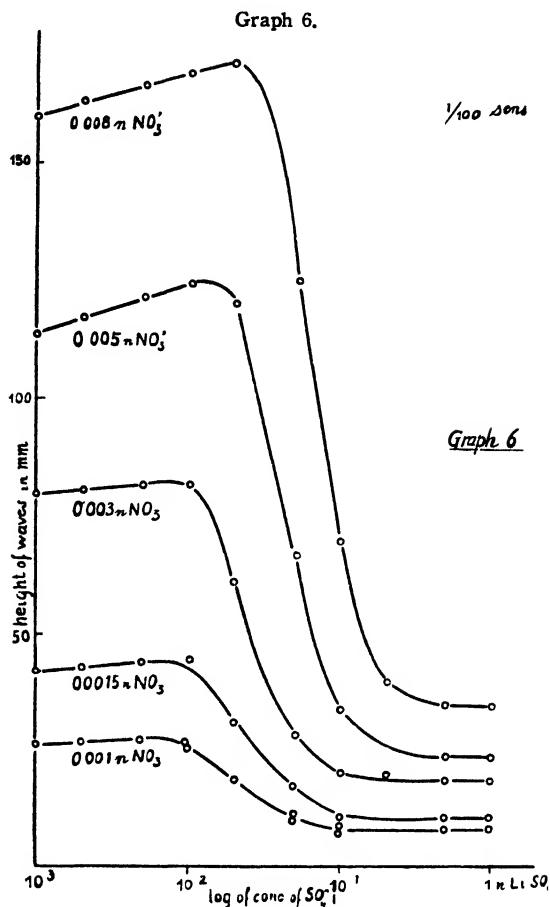
Non-reducible high-valency anions, such as sulphate, phosphate, and oxalate anions, have a remarkable effect on the reduction potential and on the height of the limiting current of nitrates and nitrites. On the other hand, monovalent anions, as those of the halides, hydroxyl ions, acetates (polarogram 27), and formiates (pol-

Polarogram 27.

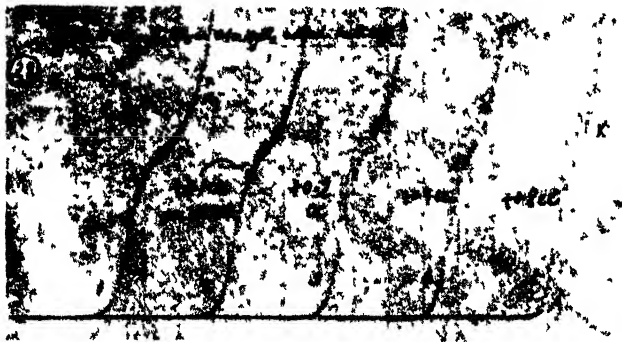


arogram 28) do not produce any marked effect on the reduction of nitrates when their concentration is varied or when they are substituted one for the other in the electrolyte of the solution.

A great change in the curve takes place when the sulphate is used, instead of the chloride of lanthanum, or when alkali sulphates are added to a solution of lanthanum chloride. Polarogram 29 shows the change of the nitrate wave into an indistinct small diffusion current, when a solution of 0.1 n.

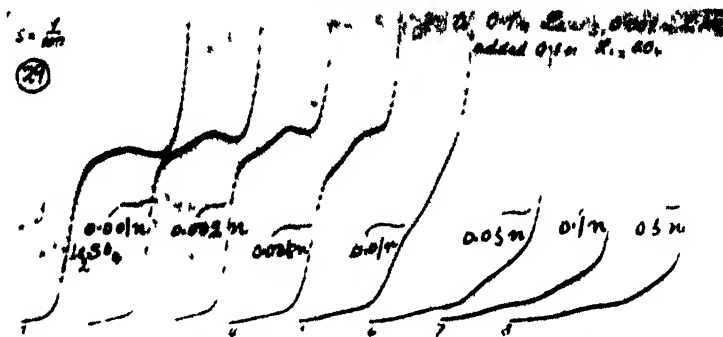


Polarogram 28.



lanthanum chloride containing 0.001 n. lithium nitrate is made about 0.05 n. lithium sulphate. See also polarogram 30 with 0.004 n. lithium nitrate in 0.1 n. lanthanum chloride.

Polarogram 29



Polarogram 30



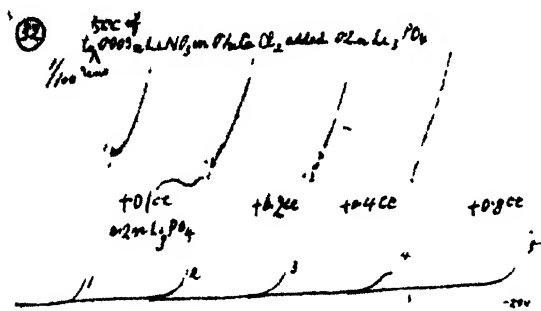
Polarogram 31



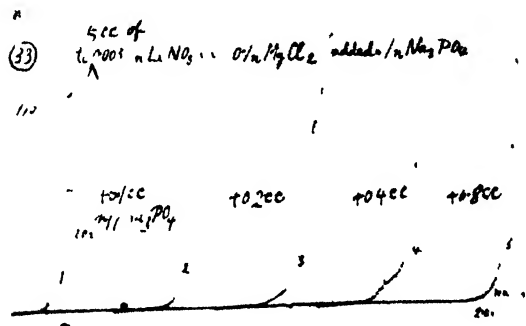
POLAROGRAPHIC DETERMINATION OF NITRATES AND NITRITES 241

The depressing influence of the sulphate ions on the nitrate wave is thus more pronounced at greater concentrations of the nitrate.

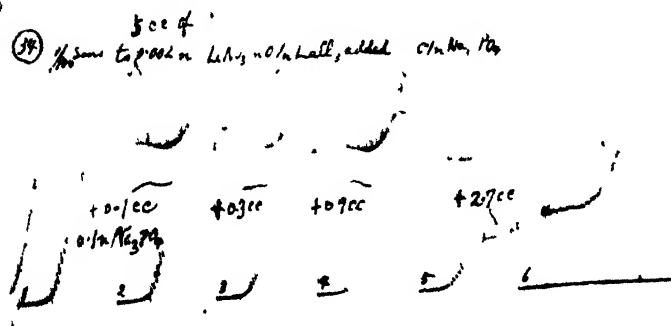
Polarogram 32.



Polarogram 33.

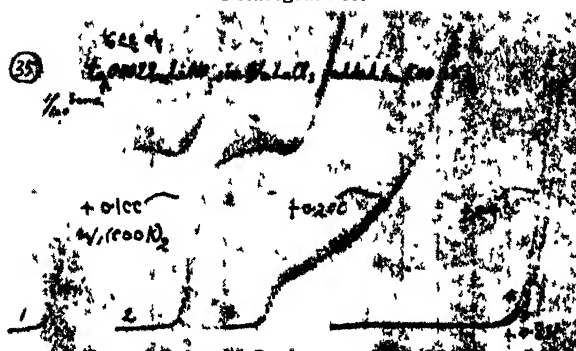


Polarogram 34.



This is shown graphically in graph 6. To produce a depressing effect, the sulphate ion concentration has to be about three times as great as

Polarogram 35.



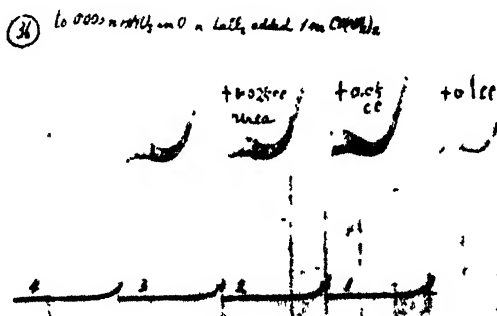
that of the nitrate.

It is interesting that in small concentrations the sulphate ion should produce even a slight increase of the nitrate wave.

A similar effect as that due to sulphates is found with a colloidal solution of barium sulphate, shown in polarogram 31. Also the oxalate and phosphate anions show this suppressing action on the nitrate waves. (Polarograms 32 to 35).

Considering the known reaction between nitric acid and urea, some experiments were also carried out to see if urea had an influence upon the nitrate wave in lanthanum chloride and strontium chloride.

Polarogram 36



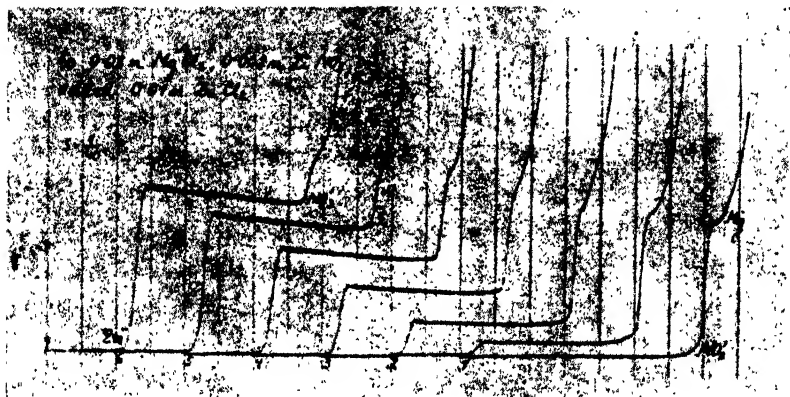
As may be seen from polarogram 36, the influence of urea upon nitrate reduction is nil.

IV. THE INFLUENCE OF CATIONS WITH MORE POSITIVE REDUCTION POTENTIALS THAN NITRATES UPON NITRATE REDUCTION

This influence was investigated by adding an 0.01 n ZnCl₂ solution, which contains 0.02 n MgCl₂ and 0.001 n LiNO₃, to 10 cc of 0.02 n

MgCl₂ solution containing 0.001 n LiNO₃. The volume of the added solution of zinc chloride solution is 0 cc, 0.5 cc, 1.5 cc, 3.5 cc, 6.5 cc and 10.5 cc respectively. This experiment was made in an hydrogen atmosphere, starting from 0.5 v. The result can be seen in polarogram 36a. It illustrates a diminution of the nitrate wave, when zinc chloride

Polarogram 36a



is added in increasing amounts. The same influence of other cations was also observed, when to a solution containing nitrate and lanthanum chloride some chloride of copper, thallium or lead was added. On the contrary, the electro-reduction of oxygen, which occurs also at a more positive potential than that of nitrate, does not produce any effect on the limiting current of nitrate reduction.

The influence of those metal ions upon the limiting current of nitrate reduction can be explained by the following theoretical consideration.

Supposing i be the intensity of the limiting current due to the electro-deposition of the cation; then $i \cdot \frac{v}{u+v} \cdot \frac{1}{F}$ gram-anions per second migrate away from the surface of the cathode and $i \cdot \frac{u}{u+v} \cdot \frac{1}{F}$ gram-cations per second reach the cathode (u and v being the ionic migration velocities). In order to deposit i/F gram-cations per second, $\frac{1}{F} (i - i \cdot \frac{u}{u+v})$ cations and as many anions must diffuse to the cathode. The number of anions, diffusing towards the cathode,

$i \cdot \frac{v}{u+v} \cdot \frac{1}{F}$, is thus exactly counterbalanced by the number of anions migrating away. Hence no anion can stay at the cathode to be reduced there.

If, on the other hand, the reduction-potential of the anion is more positive than that of the cation, electro-reduction of the anion takes place even when one single salt is present in solution. Additions of other electrolytes to this solution may lower or raise the height of the limiting current due to the electro-reduction of the anion according to whether the reduction-potential of the added ion is more positive or more negative than the reduction-potential of the anion. In the former case, a limiting current ensues before the wave due to the reduction of the anion and thus the drop in the potential across the solution, i. r., is increased; the rate of migration of anions away from the cathode is increased hereby and thus the limiting current of the anion lowered. In the latter case, when the added ions are reduced at a higher voltage than the anion, the anionic migration is partly carried on by the anions of the added electrolyte and the rate of migration of the reducible anions passing away from the cathode is, therefore, lowered; this produces a larger rate of reduction of anions, and thus their limiting current is increased.

V. THEORETICAL CONSIDERATION OF THE RESULTS

The reduction of nitrate and nitrite ions is not the only reduction of anions on the dropping mercury cathode. The anions of amphoteric hydroxides, many complexes such as cyanide, thiocyanide and organic complexes, and also anions of acids,²⁴⁾ as arsenite, selenite, bromate and iodate ions are reducible while the sulphate, phosphate, chlorate and other anions could so far not be reduced. In the case of the reducible anions outside of nitrates and nitrites, the conditions for reduction are much simpler because no excess of other cations is necessary, e. g. bromates²⁵⁾ are reduced at a potential of about -1.6 v. from solutions of potassium bromate. However, there is a certain

connection between the reduction of bromate ions and that of nitrate ions since, in the presence of multivalent cations, the reduction-potentials are shifted to more positive values. In 0.1 n. lanthanum chloride, this shift amounts to more than 0.9 volts for the reduction of the bromate ion, which compares very favourably with the shift of the nitrate reduction-potential in lanthanum chloride as against that in tetramethyl ammonium chloride. On the basis of this connection, one may conclude that the mechanism of the reduction of anions on the mercury dropping cathode in one case is the same in all cases.

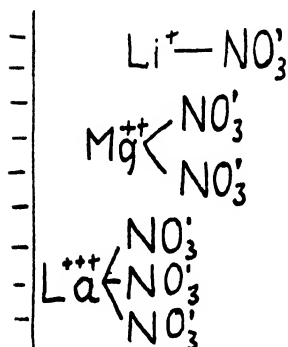
J. HEYROVSKÝ has advanced a theory of these phenomena as has been pointed out by the present author in his paper on nitrate and nitrite reduction.⁸⁰⁾

There it is regarded as impossible that—in view of the large repulsive forces between a negatively charged electrode and an anion—this could directly touch the cathode to receive electrons there; moreover, it has to be taken into account that the reduction of anions takes place only in the presence of di- or trivalent cations. The explanation is that nitrate and nitrite anions in the strong electric field existing in the close proximity of the cathode are split into the constituting elementary ions, the cationic part of which is electro-reduced. The approach of these anions to the vicinity of the cathode is facilitated by the high-valency cations which are supposed to be adsorbed at the cathodic interface. The picture of such a reduction is then as follows: In the closest proximity of a polarized cathode, there is a sudden drop of potential extending over a narrow layer, not more than an atomic diameter thick, due to the HELMHOLTZ double layer; yet deeper into the solution extends a further drop of potential, much less steep and extending over several molecule layers, which is usually denoted as the “adsorption” or FREUNDLICH’S “electrokinetic” potential, ζ . There is thus an electric field in the proximity of the cathode into which cations are drawn, dragging behind them their partner anions. The adsorption of monovalent cations, caused by the “adsorption” potentials, is by no means so

great as the adsorption of di- or trivalent cations (see Fig. 1), which latter are able to be adsorbed in a larger number and more deeply to the cathodic interface.

Consequently the nitrate or nitrite anions are drawn the nearer

Fig. 1.



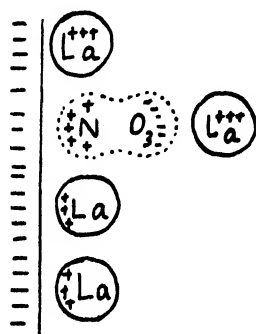
to the surface of the cathode, i.e. the deeper into the intensive electric field extending around the cathode, the higher the valency of the cations present. The effect of the strong electric field on the nitrate or nitrite ion is to tear it into the elementary cations N^{++++} or N^{+++} (see Fig 2), which at once receive from the cathode the requisite number of electrons, and oxygen anions, that join with water to form hydroxyl ions. The freshly

formed particles N^{---} instantly join the cations present to form nitrides and may undergo hydrolysis to ammonia.

This view, based on the preferential adsorption of higher valency ions, is supported by the present author's observation, that the addition of sulphate ions etc. hinders the reduction of nitrates, causing the disappearance of the nitrate wave on the curve. The divalent sulphate anions expel, no doubt, the nitrate anions from the proximity of the cathode being preferentially adsorbed to their high-valency cations.

This theory was further developed by J. HEYROVSKÝ⁽⁴⁰⁾, who applied the idea of deformability of the anions. According to FAJAN'S⁽⁴⁰⁾ description, anions which are likely to be but slightly deformable—like sulphate, carbonate and other ions—are not electro-reducible, while deformable or polarizable particles—like bromate and iodate ions—are reduced at the dropping mercury cathode, where only primary (i. e. instantaneous) reduction

Fig. 2.



can take place in order to show the effect on the current-voltage curve.

The higher the valency of a cation, the more is it able to deform anions in its proximity. Thus, in excess of divalent or trivalent cations, nitrate or nitrite anions are deformed to such an extent that they can penetrate into the electric field at the negatively charged interface. The deformation of the anion effects a displacement of the positively charged nitrogen from the negatively charged oxygen atoms (see Fig. 2). In this deformed condition it is attracted to the negatively charged interface, being in the electric field of the electro-kinetic potential, ζ . The distorted particle may now touch, at its positive pole, the negatively charged electrode, at which instant its di-pole is under such a stress that the particle breaks into N^{++++} and $3O^{--}$. The former at once receives the requisite number of electrons to form—for an instant— N^{---} ; however, these particles quickly join the lanthanum cations forming lanthanum nitride (LaN), which is hydrolyzed to lanthanum hydroxide and ammonia. The particles O^{--} , which are dehydrated hydroxyl ions, join water to form $2OH^-$. The net result is then expressed by the equation:

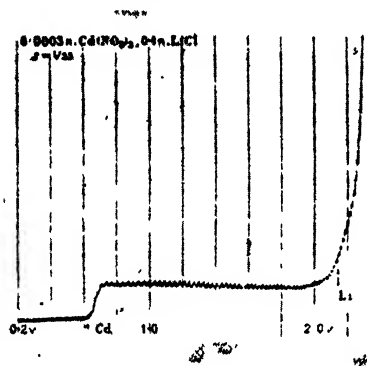


The mechanism of the electro-reduction of a nitrite anion is, of course, the same. Arsenite anions have also been found reducible—when in an alkaline solution—in the presence of barium ions, undergoing, no doubt, the above type of reaction. On the other hand, particles like bromate and iodate ions¹⁰, and more complex anions are electro-reducible even in the presence of monovalent cations. Such particles are more easily deformable than nitrate or nitrite ions and hence may be easily drawn into the electric field of the negatively charged electrode. The concentration of cations, which has to be present in solution in order to render nitrate or nitrite ions reducible, is the smallest for trivalent cations, like La^{+++} or Ce^{+++} . It is larger for divalent ions, like Mg^{++} , Ca^{++} , Sr^{++} , and very large for cations of the alkali metals. Of course the cations causing deformation have to

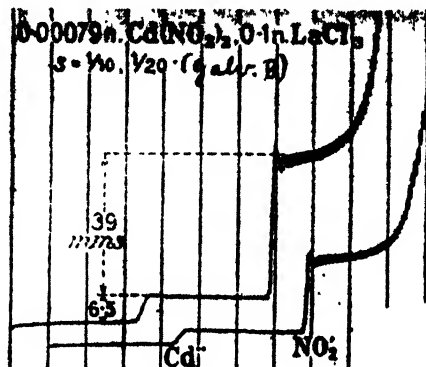
be deposited at a more negative potential than that at which the reduction of the anion takes place, otherwise they would be removed from the solution before the reduction-potential of the anions is reached. (Pol. 37).

To explain the influence of sulphate and other ions in suppressing the nitrate wave, M. TOKUOKA and J. RŮŽICKA²⁷⁾ have introduced

Polarogram 37.



Polarogram 38.



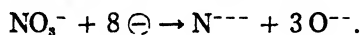
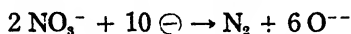
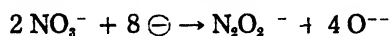
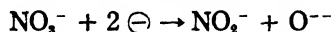
further the idea of complexes. The probable assumption of closely bound pairs of ions or complexes like $\text{La}^{+++}(\text{NO}_3)_2^{--}$, $\text{La}^{+++}\text{SO}_4^{--}$, explains how under these conditions nitrate anions are brought into close proximity of the cathode and how they are preferentially substituted by sulphate ions.

Owing to such an agency, the sulphate ions will push nitrate ions out of the sphere of the cathodic electro-reduction and a more negative cathodic potential has to be applied in order to reach their reduction.

The complexes between the sulphate and the lanthanum ions including some nitrate ions must, moreover, diffuse slowly and thus the limiting currents, which are constituted by diffusion velocity, are smaller than those observed in solutions of lanthanum chloride alone.

It was mentioned already above that the end-product of the reduction of nitrates or nitrites is ammonia. Let us now consider all the possible end-products of the nitrate reductions, viz. nitrite ion, hypo-

nitrite ion, nitrogen, and N^{3-} ion, which would be formed in accordance with the following equations:



All these cathode processes are irreversible, furthermore their potentials are not dependent only on the nitrate concentration but also on other conditions. Therefore the NERNST formula cannot

Fig 3.

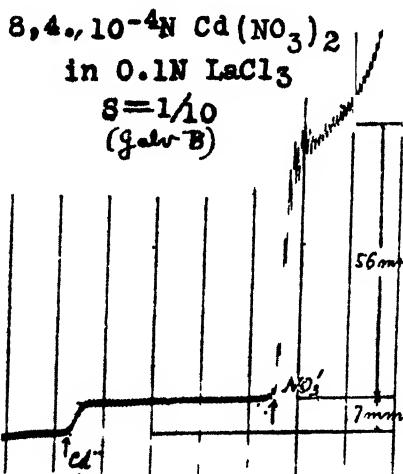


be used for calculating the shifts of potential, neither can the number of faradays used per mol. by employing this equation be calculated.

If we assume that only one end-product is formed and not a mixture, we can easily determine which end-product is concerned because the height of the wave is determined by the quantity of electricity necessary for complete reduction. This method has been described by the present author already in a previous paper²⁶⁾ where the heights of waves due to cadmium and nitrate reduction from equivalent solutions were compared. To a 0.1 n. solution of lanthanum chloride directly a solution of cadmium nitrate was added (polarogram 38a). In order to avoid disturbances due to oxygen, the solution was treated with a stream of hydrogen before the electrolysis. Then the solution was polarographically examined using 1/20 sensitivity of the galvanometer. Cadmium and nitrate ions are

here in equivalent concentrations and we know that for the deposition of one equivalent cadmium one faraday is necessary. Then the ratio of the heights of the nitrate and cadmium waves gives us directly the number of faradays used for 1 mol nitrate. The cadmium wave was 7 mm high and that due to the nitrate reduction 56 mm;

Polarogram 38a.



this means that NO_3^- needs 8 times the quantity of electricity for one gram-equivalent as cadmium. In other words, 8 faradays are needed for a complete reduction of one gram-ion NO_3^- .

It must also be mentioned here that in presence of a large excess of indifferent electrolyte the ratio of the ionic mobilities ($\frac{1}{2}Cd : NO_3^-$

$=46:62$) has no longer significance. The diffusion velocity with which the two ions are brought to the cathode is in both cases the same because the transport of electricity in an excess of e.g. lanthanum chloride is carried out mainly by the lanthanum and chloride ions.

The disadvantage of this method of measurement is that the nitrate wave is lowered if previously another substance is deposited, i. e. in the above mentioned case the nitrate wave is not at its limiting height because of the previous deposition of cadmium. This effect is, however, partially offset—especially in small concentrations of cadmium nitrate—by the fact that the wave due to nitrate is at a higher potential at which also the mercury drops are falling more quickly than what would ensue in a higher wave.

Due to these disturbances, the ratio of NO_3^- wave to $\frac{1}{2}Cd^{++}$ wave is not always 8 as is shown in Table IX.

Table IX.

Height of Cd^{++} wave	Height of NO_3^- wave	ratio
7 mm	56 mm	8.0
2 mm	15 mm	7.5
8 mm	61 mm	7.6
6 mm	45 mm	7.5
7 mm	52 mm	7.4
5 mm	38 mm	7.6

From these results it becomes evident that the number of faradays necessary for the reduction of 1 gram-equivalent NO_3^- is always more than 7; on the average 7.6. We can conclude therefore that if no wave appears before the nitrate wave, it would be higher and corresponding to 8 faradays.

If 8 faradays are used in the reduction, it is evident that only the last of the above mentioned equations is applicable, or:



Therefore the end-product must be ammonia.

The same experimental procedure and theoretical reasoning was also used in the case of nitrites and it was found that 6 faradays were necessary for the complete reduction of one gram-equivalent of nitrite, according to the following equation:



This is demonstrated by polarogram 38, where to 0.1 n. lanthanum chloride 0.00079 n cadmium nitrite was added:—the cadmium nitrite being prepared for this purpose in a specially pure state according to the prescription given in the literature²⁸). The ratios of the heights of the waves due to the reduction of nitrite and cadmium ions from their equivalent solutions are $39/6.5=6$. Thus also nitrites in the presence of lanthanum chloride are reduced to ammonia on the dropping mercury cathode.

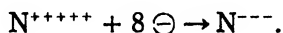
According to the number of faradays used, the ratio of the limiting waves of nitrate and nitrite should be 8:6, i. e. the nitrate

wave should be 1.23 times as large as that of the other anion. This is in agreement with the formerly obtained results (see discussion of nitrite reduction in this paper), where the heights of the waves due to nitrate and nitrite reduction were compared and 1.27 was found as the result.

Before the reduction of the nitrate ion is possible at the mercury dropping cathode, the nitrate anion must be deformed and torn apart in the electric field in the close proximity of the cathode surface:



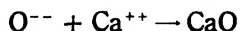
This five-valent nitrogen cation is attracted to the cathode and at once obtains 8 electrons to form the trivalent nitrogen anion:



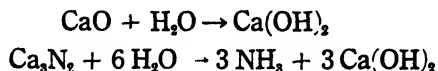
This N^{---} anion is very unstable and at once reacts with a cation which is present on the mercury surface, and the corresponding nitride is formed, e. g.:



Similarly the hypothetical O^{--} anion can react:



Both these compounds are hydrolyzed in water and the end-products are ammonia and hydroxide, according to the equations:



This same mechanism of course also holds good for the nitrite reduction, the only difference being the number of faradays used. The reduction-potentials of nitrates and nitrites are the same under the same conditions; they are only influenced by the valency and the adsorbability of the added cations in the presence of monovalent anions. The values of the potentials for 0.002 n. nitrate are given in Table X.

Table X.

solution	valency of cation	π in v.	average	difference	
0.1 n. LaCl_3	III	-1.22	-1.23	0.54	0.93
0.1 n. CeCl_3	III	-1.23			
0.1 n. MgCl_2	II	-1.74	-1.77	0.39	
0.1 n. CaCl_2	II	-1.78			
0.1 n. SrCl_2	II	-1.79	-2.16	0.39	
0.1 n. $(\text{CH}_3)_4\text{NCl}$	I	-2.15			
0.1 n. LiCl	I	-2.17			

If in the solution instead of monovalent anions higher valent anions are present, the function of cations on the reduction potential is decreased. This may be explained in agreement with the above theory on the assumption that with higher valency also the adsorbability of the anions becomes larger. In this case anions are adsorbed together with the cations to the cathode surface and the electric field of adsorption will not be so strong as when only cations and monovalent anions are in solution. Only if a greater difference of potential is applied to the electrodes, the electric field reaches such a value that a reduction can take place. For a 0.002 n. nitrate solution the reduction-potential was thus found as -1.58 v. from 0.1 n. lanthanum sulphate solution as against -1.22 v. from 0.1 n. lanthanum chloride; further -1.82 v. from 0.1 n. magnesium sulphate solution as against -1.74 v. from 0.1 n. magnesium chloride. In the first case, the sulphate ion caused a displacement of 0.36 v. and in the second case of 0.08 v. to more negative values.

These negative displacements are not the same in both cases and we have to assume therefore, as an explanation, that in the case of lanthanum, complexes with sulphate ions are formed which weaken the electric field more than free sulphate anions in the case of magnesium which has no complex formation.

Let us compare further the heights of the waves due to 0.002 n. tetramethyl ammonium nitrate, obtained by electrolysis with the dropping mercury cathode, in 0.1 n. solutions of chlorides containing

various cations (Fig. 3). One can see that the waves have only the same height when the cations have the same valency. This phenomenon appears not only in the case of nitrates but also in the case of manganese—as has been shown in another paper²⁷⁾. Here, however, the effect is the inverse of that with nitrates, the trivalent cations depress most, the diffusion current of manganous ions while the diffusion-current of nitrate ions reaches its largest limit in their solutions. These phenomena are however not exact, neither with cations nor with anions and therefore no theoretical conclusions can be drawn.

Finally, for theoretical studies, the sharp bend in both reduction waves is especially interesting which is formed best from 10^{-4} to 3×10^{-3} n. concentrations of nitrates or nitrites, if in the electrolyzed solution lanthanum chloride and traces of acid are present. As oxygen prevents this phenomenon, the discontinuity only appears in solutions which formerly were freed from atmospheric oxygen by a stream of hydrogen; when electrolyzing on air, the discontinuity may be also brought about by increasing the hydrogen ion concentration. On the assumption that atmospheric oxygen, dissolved in the electrolyte, causes an alkaline reaction in the proximity of the cathode after it is reduced, we may conclude that the discontinuity is caused by hydrogen ions. If we remove the atmospheric oxygen from the solution, the neighborhood of the cathode becomes acid, because the pH of a lanthanum chloride solution is on the acid side. In this case the ions La^{+++} and H^+ move together to the cathode; as however the latter have a greater diffusion-velocity, they occupy the largest part of the cathode surface. By this the field, causing the adsorption of lanthanum ions, is diminished and its strength is not sufficient to tear apart the nitrate ions. To bring this about, the E. M. F. has to be increased to a potential where the reduction of hydrogen ions takes place; by this they are removed from the surface of the cathode and lanthanum ions are adsorbed. The decomposition of nitrate ions, like the reduction of N^{++++} to N^{--} is extremely rapid so that the current-intensity suddenly increases.

If atmospheric oxygen is present in solution, the hydrogen ions

in the lanthanum chloride solution are neutralized and only lanthanum ions can pass to the cathode. By increasing applied E. M. F. the concentration of lanthanum ions increases slowly on the cathode surface and accordingly the polarographic wave of nitrate shows a gradual increase.

VI. POLAROGRAPHIC DETERMINATION OF NITRATES, NITRITES AND THEIR SEPARATION

If it is desired to employ the polarographic method for the analytical determination of nitrates or nitrites, at first the sample under investigation must be brought into a solution which should contain no components which could influence the height of the reduction-wave. The first condition is the acidity of the solution, which should be between pH 5 and pH 7. If the solution is more acid, the reduction-wave is displaced to that of hydrogen ions and its height does not indicate the true concentration of nitrate ions because the reduction of hydrogen also contributes to the current intensity. On the other hand, the pH should not be larger than 7, because then lanthanum hydroxide is formed and we have in solution undissociated molecules or precipitates of lanthanum hydroxide. A similar condition is that the solution should not contain substances which form either insoluble salts with lanthanum ions, as phosphates and oxalates, or undissociated salts, as sulphates. Finally the quantitative determination is also disturbed or hindered by a large concentration of any cations or anions which deposit or are reduced at the dropping mercury cathode at more positive potentials than nitrates.

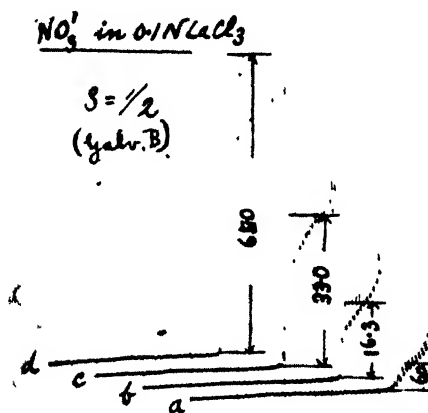
A. Determination of Nitrates

If nitrates are determined qualitatively or quantitatively from solutions containing no components which influence the reduction, the sensitivity of the polarographic method is very large. In our case it is still larger than in other determinations of cations, as the quantity of electricity is 8 times greater with nitrates and 6 times

with nitrites than in the case of ordinary deposition of cations. Therefore, the waves due to the electro-reductions are in the first case 8 times and in the second case 6 times as high as the wave due to the deposition of cations, which take only one faraday for its deposition.

The possibility of accurate determination of nitrates with the low sensitivity of galvanometers has already been ascertained in Chap. II.

Polarogram 39a.



Here is shown an example of the nitrate determination with the high sensitivity of the galvanometer. For the indifferent electrolyte 0.1 n. lanthanum chloride (Merck puriss.) was used, and for nitrate the solution of lithium nitrate was used. The experiment was carried out in the atmosphere of hydrogen with 1/2

sensitivity (Galv. B). The result is shown on polarogram 39a and Table XI.

Table XI

Curve	Conc. of NO_3^- in 1.10^{-6} n.	Height of waves in mm.	Height of waves for 1.10^{-5} n. NO_3^- in mm.
a	18.	6.0	3.33
b	46.	16.3	3.54
c	92.	33.0	3.58
d	184.	65.0	3.53

mean value 3.50 ± 0.06

The height of the wave for the unit concentration of nitrates remains almost constant, the error of which is only $\pm 1.71\%$. It can

be seen, that the nitrate is easily and properly measurable and grows proportional to the concentration of the nitrate. On the assumption, that we can measure the wave height as far as 0.1 mm, it is possible to determine the nitrate concentration of $3 \cdot 10^{-7}$ n. with 1/2 sensitivity of this galvanometer. If a galvanometer with higher sensitivity would be used, the sensibility of the polarographic method for nitrate ions could be more increased. Often in practise however, the sensitivity of the polarographic method is limited by various influences due to the components in the solution.

For the determination of nitrates, the concentration of which is smaller than $2 \cdot 10^{-3}$ n., 0.1 n. solutions of lanthanum chloride is sufficient.

Such a La^{+++} concentration is not sufficient, as has been written above, to bring about a complete reduction of nitrates if their concentration is greater than 2×10^{-3} n. But even in this case the wave is easily reproducible, i. e. the height of the wave is constant if the same lanthanum chloride solution is used. Of course it is necessary to employ always the same apparatus, sensitivity of the galvanometer, distance of the galvanometer from the photographic paper and the same dropping capillary (i. e. one with the same drop-time etc.) as all these factors would change the height of the curve if they were not constant.

If we fulfill the above mentioned requirements, the determination of nitrates and nitrites can be very much simplified by first making a calibration curve. For the construction of such a curve, the same solution of lanthanum chloride is used which will be employed in our further work; to this we add exactly measured solutions with known concentrations of nitrates and nitrites which have been prepared from non-hygroscopic salts (in our case sodium nitrate prepared from Merck, and barium nitrite from Kahlbaum were used).

In addition to these calibration curves of nitrates and nitrites, a further calibration curve for nitric oxide has to be constructed for the purpose of separating the two anions (curves from a 0.1 n. hydrochloric acid solution are best). The heights of the waves at a

definite sensitivity of the galvanometer are measured and the values plotted on the Y-axis of a graph, while the X-axis represents the concentration either expressed in normality, or in % of anions, or directly in % nitrate. Similar graphs have been presented already in the experimental part of this paper (graphs 2, 4 and 5), but for analytical purposes they are not quite sufficient and have to be drawn on a larger scale. Mostly calibration curves were used which were constructed only from zero up to a concentration of 3×10^{-3} n., so that 1 cm of the X-axis represented a 10^{-4} n. concentration and 4 mm of the Y-axis were equal to 1 mm of the height of the wave. Experimental points were then connected by a narrow line so that the whole diagram allowed a reading to 10^{-5} n. concentrations.

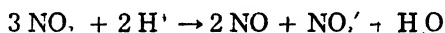
B. Separation of nitrates and nitrites

Both anions cannot be distinguished polarographically in neutral solutions. For a separation one must therefore make use of the instability of nitrites in acid medium and the reducibility of the formed nitric oxide.

A typical separation is demonstrated on polarogram 39. Here 0.1 n. lanthanum chloride was used as indifferent electrolyte, with which first a trial curve 1 was carried out; it could thus be determined that at a sensitivity of 1/100 no impurities would interfere. The curve starts at -1.0 v. applied E. M. F. and the bend at -1.9 v. already belongs to the deposition of lanthanum. Between both these values no wave appears which could disturb our determination. The wave on curve 2 is caused by the reduction of 0.0022 n. sodium nitrate which had been added to the solution, its height is 56.8 mm. The next curve (3) was obtained after 0.0021 n. barium nitrite had been added to the pure lanthanum chloride solution instead of the nitrate. The wave due to the nitrite reduction is 46.4 mm high. After this an experiment was carried out for which exactly 5 cc of the solution, with which curve 2 was taken, and 5 cc of the solution (curve 3) was mixed. The ensuing solution was then electrolyzed

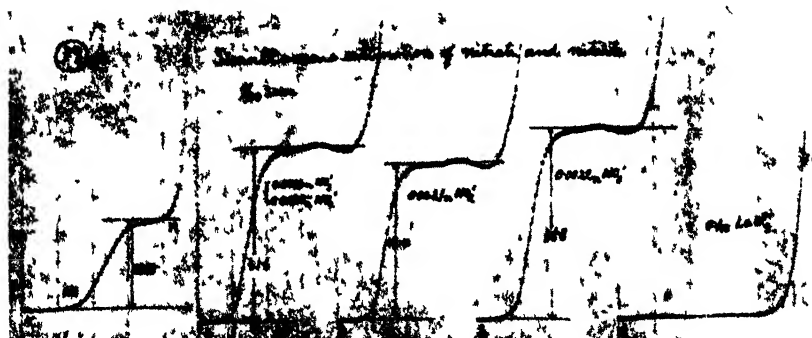
and curve 4 obtained, which is caused by the joint reduction of nitrate and nitrite ions and is 51.6 mm high. This height is the mean of the two formerly obtained waves where each anion was separately reduced, viz. $56.8 + 46.4 = 103.2/2 = 51.6$, which should be expected as each ionic species is now present in only one half of its former concentration.

If we now assume that the respective concentrations of nitrate and nitrite ions are unknown, we have to add concentrated hydrochloric acid (in our case 1 drop of 11 n HCl to 10 cc electrolyte is sufficient) in order to keep the concentrations of nitrate and nitrite practically unchanged and to bring the concentration of hydrogen ions to about 0.1 n. Then the solution is electrolyzed again (see curve 5, starting at -0.6 v). Here a wave appears, 26.75 mm high, which is due to the reduction of nitric oxide. From the calibration curve (similar to graph 5) a nitric oxide concentration of 7.0×10^{-4} n. could be read off for 1/100 sensitivity of the galvanometer. If we consider that in accordance with the equation



a nitric oxide concentration is caused equal to 2/3 of the nitrite concentration, then we simply multiply the found concentration of nitric oxide by 3/2 to obtain the concentration of nitrite ions. In our case the calculated concentration is 1.05×10^{-4} n. From the calibration curve similar to Graph 4) for nitrites we can read off

Polarogram 39



the corresponding height of the wave in lanthanum chloride solution to be 23.2 mm. By subtracting this value from the obtained height of the wave due to both ions (51.6 mm) we get that due to nitrate ions alone, viz. 28.4 mm. The corresponding concentration of nitrates is obtained from the respective calibration curve and found to be 1.10×10^{-3} n. Thus the calculated results are seen to be in close agreement with the actual known values.

If the procedure is followed accurately, the reproducibility of the reduction curve is 100% and the accuracy of the determination then only depends on the exact measurement of the height of the wave and on the precision with which the graph was prepared.

For control purposes we can further check our results by passing for some time a stream of hydrogen gas through the acidified solution (curve 5) which removes the nitric oxide. After neutralizing the solution we electrolyze it once more and obtain a curve with the nitrate reduction. From the concentration, thus found directly, we have to subtract the quantity of nitrate ions which has been formed by the decomposition of nitrite ions; by this we obtain once more the concentration of the original nitrates.

C. Determination of nitrates in the presence of sulphates

In the experimental part it has been mentioned already that sulphate ions hinder the reduction of nitrates. If the sulphate ion concentration is three times larger than that of nitrate ions (or even more) the reduction-wave due to nitrates is reduced to about one fourth of its normal height and is badly measurable, so that a quantitative determination is quite impossible. Therefore in a sample containing sulphates besides nitrates (resp. nitrites) these anions can only be determined qualitatively. For a quantitative determination it is absolutely necessary to remove the sulphate ions before electrolysis. As usual barium ions are used for the precipitation which has the further advantage that an excess of barium has no influence on the reduction, so that also in this case the polarographic deter-

mination is not very difficult. From the following we can see, however, that the accuracy of our method is somewhat hampered by this operation.

In a series of experiments, this sulphate influence was especially studied. A solution containing nitrate ions was polarographically examined; then sulphate ions were added, the solution heated and a solution of barium chloride added by drops until all of the sulphate was precipitated. After this, the solution was filtered and the filtrate brought up to twice the volume of the original nitrate solution by adding water and enough lanthanum chloride to make the solution 0.1 n. LaCl_3 . After cooling, the solution was electrolyzed and a new curve taken, which differed somewhat from the original curve. The results of these experiments are given in Table XII.

Table XII.

$\frac{1}{2}$ NO' before addition of SO_4''	SO_4''	NO' after ppt. the SO_4''	error in %
0.00051 n.	0.001 n.	0.00054 n	+ 5.9
0.00050	0.01	0.00053	+ 6.0
0.00053	0.1	0.00055	+ 3.8
0.00104	0.001	0.00109	+ 4.8
0.00123	0.001	0.00128	+ 3.2
0.00116	0.02	0.00121	+ 4.3
0.00105	0.1	0.00110	+ 4.7
0.00109	1.	0.00116	+ 5.5
0.00253	0.01	0.00261	+ 3.2
0.00245	0.1	0.00252	+ 2.9
0.00257	1.	0.00264	+ 2.9

According to these results, the final concentration of nitrate ions appears always larger, the average error is 4.3%. From this it follows that the source of error here cannot be an occlusion of nitrate ions in the barium sulphate precipitate but must be the influence of traces of sulphate* ions which are still present in the solution after

* 2.07–2.53 mg BaSO_4 soluble in 1L. Water at 18°C (Seidell, Solubilities of Inorg. & Org. compds, p. 1084)

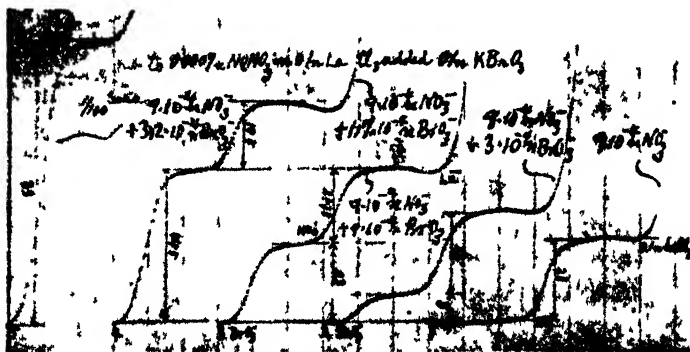
the precipitation, because as has been mentioned in the experimental part of this paper—the presence of small traces of sulphates increases somewhat the nitrate wave (graph 6). One must be careful that no colloidal barium sulphate shall be formed during the precipitation, otherwise the height of the wave would be diminished as is shown in polarogram 31.

Other substances, which have to be removed from the solution before a polarographic determination, due to their disturbing action upon the nitrate wave, are phosphoric acid and such multivalent acids as oxalic, citric and similar acids.

D. Determination of nitrates in the presence of bromates and iodates.

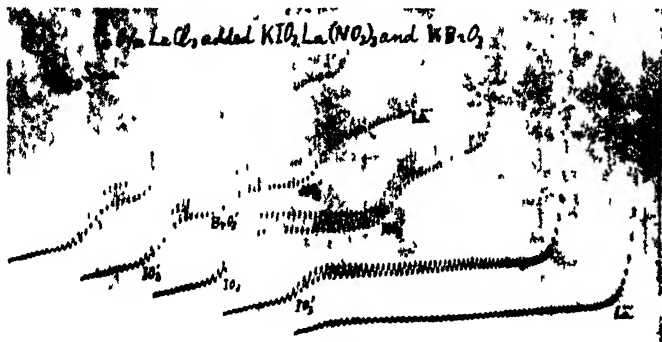
It should briefly be mentioned that a few experiments were carried out on the separation of nitrates, bromates and iodates. Here polarograms 40 and 41 may serve as examples. It can be seen how

Polarogram 40



different the reduction-potentials of all these anions in 0.1 n. lanthanum chloride solution, so that a qualitative separation is easily effected. For quantitative purposes the polarographic method is only suitable if traces of bromate and iodate ions are present, while the concentration of nitrates is considerable. If the reverse is the case, the wave due to nitrate reduction is covered by a bromate or iodate

Polarogram 41



wave which appears before on the polarogram. As the concentration of bromate ions becomes larger the height of the nitrate wave becomes smaller.

E. Determination of nitrites.

All that has been written concerning the nitrate determination is of course, also valid for that of nitrites, if we wish to carry it out in lanthanum chloride. Much simpler however, is the determination from an acid medium, because the anions (SO_4^{2-} , PO_4^{3-} etc.) have no influence upon the height of the nitric oxide wave. In spite of this, certain conditions have to be fulfilled, especially if only small quantities of nitrite are present. In this case, always the atmospheric oxygen has to be removed before the electrolysis, as it might disturb the curve. During the removal of oxygen by a stream of hydrogen gas, the solution should never be acidic, as otherwise simultaneously the nitric oxide is removed by the hydrogen. Therefore always first the pH of the solution has to be ascertained; a weakly alkaline solution is desirable. Only after the oxygen has been completely removed the hydrochloric acid (free from O_2) is added from a burette until the hydrogen ion concentration is 0.1 n.

By this simple method, nitrites may be determined in any mixture or compound. The only exception is here the case where nitrate ions are present in a large excess. After the addition of hydrochloric acid

a very large concentration of nitric acid is brought about and a few additional circumstances hinder the determination. In the first place, at such a high concentration of nitric acid, much mercury dissolves from the anode in the form of mercury ions which are deposited again at the cathode at the beginning of the electrolysis, so that a very large wave is obtained at the start on the polarograms and only very small sensitivities of the galvanometer can be used. Furthermore the hydrogen from nitric acid deposits at a more positive potential so that the nitric oxide wave coincides with that due to the deposition of hydrogen ions. In this case we have to employ a slower method which is based on the fact that the nitric oxide liberated from the nitrite ions is carried out with the hydrogen or nitrogen bubbling through the solution into a saturated solution of calcium hydroxide. Here the nitric oxide combines and later can be polarographically analyzed after the solution has been acidified. The bubbling should not be too fast in order to bring about a quantitative reaction of the nitric oxide with calcium hydroxide. For 10-20 cc of solution about 10-15 hours are required to remove all the nitric oxide quantitatively.

F. Nitrate determination in fertilizers.

As a practical application and proof of the suitability of the polarographic determination of nitrates, several samples of fertilizers containing nitrogen in the form of nitrate were analyzed. In the first group only calcium was present besides nitrates, in the second ammonium ions and others, while the third group also contained phosphate ions. To check the polarographically obtained results, parallel with most samples also a volumetric determination was carried out. As in the polarographic method only nitrogen which is bound as nitrate, can be determined, the free ammonia had to be removed from the samples before analysis by the volumetric method. For this reason the polarographic method already proves superior because it takes a shorter time to carry out an analysis and the ammonia does not first have to be removed.

Before each analysis, the sample was homogenized and dried in a desiccator over calcium chloride. For a polarographic determination 1-1.5 gr of the sample was weighed out according to the suspected amount of nitrate. It was dissolved in warm water and the volume then brought to 10 or 100 ccs so that the concentration of nitrate was about 0.1 n. In cases where the sample contained no components which have an influence on the reduction, the pH of the solution was determined with Merck's Universal Indicator and eventually brought to pH between 5 and 7 by adding hydrochloric acid or sodium hydroxide respectively. After this the solution was added to 10 cc of 0.1 n. lanthanum chloride and electrolyzed. For control purposes always first a curve was taken of the pure lanthanum chloride alone and then 2 to 4 curves were taken with various additions of the prepared solution of the fertilizer (The additions were made from a microburette, 0.05-0.2 cc). By this means an average of 4 results could be obtained and thus errors eliminated which might be due to inaccurate additions. If the investigated sample contains phosphates (qualitatively tested in the usual way), they must be removed before electrolysis in any way. As phosphate ions form insoluble compounds with the lanthanum ion, we should have to add a very large amount of lanthanum chloride in order to first precipitate all the phosphates present and further to have enough of an excess so that the solution would still be 0.1 n LaCl_3 . This procedure, however, is not satisfactory as the precipitation of phosphates by lanthanum is not complete, especially not in acidic solutions which have been mostly investigated here. Therefore, the usual method for the precipitation of phosphate ions as magnesium ammonium phosphate has here been employed. The experiments were carried out as follows: to acidified solutions, containing a mixture of magnesium chloride and ammonium chloride in excess, besides the phosphate was added in drops a solution of ammonia—after the mixture had been heated to boiling point—until free ammonia could be smelled. After cooling, the magnesium ammonium phosphate was filtered off and the free ammonia in the filtrate neutralized by hydrochloric acid. After this opera-

tion we have in solution magnesium and ammonium ions, which have, however, no influence upon the height of the nitrate wave in lanthanum chloride.

For the volumetric determination the method of Devarda¹¹⁾ was used. In a 3/4 L flask about 0.1–0.2 gr of fertilizer was dissolved in 150 ccs water. When the sample contained free ammonia, about 10–20 cc of a 10% sodium hydroxide solution was added and about 2/3 of the total solution distilled off; thus all of the free ammonia should have been removed. Afterwards the solution was again brought to 150 cc with water and after it was cool, 2 gr Devarda alloy (Merck) was added. The flask was then at once connected with a distillation apparatus with a Péligré flask at one end. The latter contained 10 cc 0.1 n. sulphuric acid and about 10 cc of water besides 1 drop of methyl red. After one hour's time, the solution was slowly heated and 2/3 of it distilled off into the Péligré flask. The remaining free acid was titrated with 0.1 n. potassium hydroxide.

The results of both nitrate determinations are given in Table XIII, in which the nitrate concentration is calculated as % nitrogen. In the last column of this table, the relative errors of the polarographic when referred to the Devarda method are given in % nitrogen.

It is striking that the polarographic results are always lower with the exception of the case where the sample contained phosphates and the error is positive. These differences can be easily explained if we consider that the determination according to Devarda is always connected with a positive error which is caused by traces of ammonia in the analysed substance or in the absorbing acid. In the polarographic analysis, the presence of ammonia does not interfere and therefore the values thus obtained are smaller. On the basis of the above we may also assert that the polarographic method is more exact. Larger results in the polarographic analysis, where phosphates are present in the analysed solutions, may be attributed to the influence of the trace of the remainder of phosphates* in the filtrate, as in the

* 0.052 grs ammonium magnesium phosphate are Soluble in 100 grs of water at 20°C (Seidell, Solubilities of Inorg. & Org. Compounds P. 61).

Samples No	Composition	Polarographic method weighed out gr	Diss to cc	%N	Aver %	Devarda method weighed out gr.	%N	Aver %	Difference betw. polar. & Devarda methods.
1.	Ca ⁺⁺ , NO ₃ ⁻	1.0229	100	14.22		0.1023	14.28	14.30	-0.08
"	"	0.0825	100	14.20	14.22	0.1273	14.31		
"	"	0.0248	10	14.23					
2	"	0.8529	100	15.35	15.32				
"	"	0.0173	100	15.29					
3	K ⁺ , PO ₄ ³⁻ , NO ₃ ⁻	0.7017	100	14.17	14.16	0.1435	14.05	14.05	+0.11
"	"	0.0238	100	14.14					
4.	"	0.5237	100	15.63		0.1243	15.54	15.51	+0.03
"	"	0.0509	100	15.58	15.60	0.1507	15.48		
"	"	0.0481	10	15.60					
5	K ⁺ , NH ₄ ⁺ , NO ₃ ⁻	0.4845	100	7.595	7.60	0.1442	7.61	7.61	-0.01
6.	Ca ⁺⁺ , NO ₃ ⁻	0.3091	100	12.48	12.48	0.1138	12.52	12.52	-0.04
7.	K ⁺ , Ca ⁺⁺ , NH ₄ ⁺ , PO ₄ ³⁻ , NO ₃ ⁻	1.5994	100	7.76		0.2102	7.67	7.65	+0.03
"	"	1.0254	100	7.73	7.71	0.1635	7.615		
"	"	0.0381	10	7.725					
8.	NH ₄ ⁺ , NO ₃ ⁻	1.1637	100	17.04	17.07	0.1403	17.08	17.09	-0.02
"	"	1.0235	100	17.10		0.1392	17.10		
9.	Ca ⁺⁺ , NH ₄ ⁺ , NO ₃ ⁻	0.1348	10	15.28	15.28				

case of sulphates, where small concentrations of sulphate ions produce even a slight increase of the nitrate wave.

The biggest advantage of the polarographic method is the rapidity with which the determinations can be carried out. While an analysis by the Devarda method takes about 2 hours, a complete polarographic determination from the weighing of the sample to the developing of the photographic paper (polarogram) is finished in 20-25 minutes. This includes the taking of three curves at least, by which a direct control is possible. If sulphate or phosphate ions are present, the polarographic analysis is prolonged a little by the necessary precipitation.

If we compare the methods of the determining nitrates on stable electrodes with the present one, we find that the accuracy of the polarographic method is indeed satisfactory so long as solutions are analyzed which contain no complicating substances. It is to be expected that also on stable electrodes the results become less accurate if sulphates or phosphates are present, but this cannot be proved, however, as no such practical applications have been presented in the literature of the subject. The time saved by the polarographic method remains still a most impressive and favourable factor compared with the tedious method with stable electrodes where all of the nitrate ions have to be electrolyzed out of the solution.

VII. SUMMARY AND CONCLUSION

Although the electrochemical method is used widely as a rapid and simple one in modern analytical chemistry, none of the old electroreduction processes by means of stable electrodes can be used satisfactorily for the determination of nitrates and nitrites in unknown solutions. Owing to its rapidity, simplicity and accuracy the polarographic method is more suitable for this purpose. This method is especially fitted for the determination of these anions, because they take 8 or 6 faradays respectively for the complete reduction of one gram-equivalent ion, so that the sensitivity of determination in this case is 8 or 6 times that in the case of those ions, which takes

only one faraday for the reduction of one gram-equivalent ion. Starting from this point of departure the present writer undertook the investigation to ascertain the conditions necessary to get a complete manipulation for the determination of nitrates and nitrites by means of the polarographic method. First, the characteristics of the polarographic curves of nitrates and nitrites were examined in the presence of cations with different valencies, i. e., quarternary amine bases and its salts, alkali metals, alkaline earths, lanthanum, cerium, aluminium and thorium. The reduction-potentials of nitrates and nitrites are quite the same, other conditions remaining the same except in the case of thorium, in which case nitrates and nitrites have different reduction-potentials. In general, the higher the valency of the cation, the more positive the reduction potential and the higher the wave of nitrates.

So that the trivalent cations give more positive reduction-potential and higher wave of nitrates than bi- or monovalent cations. Of these three trivalent cations lanthanum is the most suitable for our purpose. The lanthanum chloride solution has the following superiority over the other two cations. In the case of aluminium the reduction-potential of nitrate is very near to the deposition potential of aluminium. With a solution of cerium chloride the maximum phenomenon often appears. The solution of chlorides of cerium or aluminium has a greater tendency to hydrolyse, which makes the height of the nitrate wave inaccurate. On the contrary, the solution of lanthanum chloride gives a very sharp and clear wave of nitrates. The hydrolytic solution of thorium tetrachloride can not be used for the quantitative determination of nitrates. According to these results lanthanum chloride is chosen as the best indifferent electrolyte for the polarographic determination of nitrates and nitrites. The best sample of lanthanum chloride is that of Merck puriss. The necessary conditions for the determination of nitrate were in detail investigated with the solution of lanthanum chloride. The following results were obtained.

1) The necessary and sufficient concentration of lanthanum ion for the maximum height of nitrate wave is 50 times that of the nit-

rate ion to be determined, otherwise the wave does not grow proportionally with the concentration of nitrate ion. For nitrite determination this number is 38-40 times the nitrite ion to be determined.

2) The hydrogen ion concentration of the solution for electrolysis must be pH 5-7. The acidity of this degree gives the polarographic curve a sharp discontinuity, which makes the measurement of the wave height easy. A larger acidity no longer shows the true height of the nitrate wave.

3) The addition of alkali or atmospheric oxygen to the solution makes the bend on the polarographic curve continuous.

4) With a low sensitivity of the galvanometer e. g., 1/100, the determination can also be carried out in the open air, because the electroreduction of oxygen does not produce any effect on the limiting current of the nitrate reduction.

5) Non-reducible high-valency anions, as sulphate, phosphate, and oxalate ions, have a marked action in lowering the nitrate wave. Monovalent anions, such as those of the halides, hydroxyl, acetate and formiate, do not show any marked effect.

6) The addition of those cations, which deposit at more positive potentials than the nitrate, diminishes the wave height of nitrates.

7) The solution to be electrolysed should not contain substances forming either insoluble salts or undissociated salts with lanthanum ions.

All the facts recognised as taking place with nitrate in neutral or alkaline solutions of di- or trivalent cations, are also valid for nitrite, except for the number of faraday necessary for the complete reduction of one gram-equivalent ion, which is 8 for nitrates and 6 for nitrites. This number of faraday means, that the end-product of reduction of nitrate or nitrite in neutral or alkaline solution is ammonia. The result of these experiments supports HEYROVSKÝ's theory concerning the cathodic reduction of nitrates and nitrites on the dropping mercury cathode in the presence of lanthanum or other cations. HEYROVSKÝ explains this cathodic reduction by the adsorption of nitrate or nitrite ion by the cations that are drawn to the dropping

mercury cathode. This adsorption is accelerated by the deformation of nitrate or nitrite ions in the presence of cations such as lanthanum, etc.

The determination of nitrites in acidic mediums, which was studied to some extent by HEYROVSKÝ and NEJEDLÝ, was further studied in detail. Although the nitrite in lanthanum chloride solution is determined with larger sensitivity, it is much simpler to determine it in acidic mediums, because sulphate, phosphate and other non-reducible anions have no influence upon nitric oxide waves. This method is applicable without any preliminary procedure to any mixture or compounds, except for the large excess of nitrate ions. In such a case nitric oxide is first replaced by hydrogen or nitrogen gas into a saturated solution of calcium hydroxide. After acidifying this solution the electrolysis is carried out.

The reproducibility of these determinations of nitrates or nitrites is almost 100%, when every necessary condition has been fulfilled, that is, the apparatus itself, the sensitivity of the galvanometers, the drop-time of the capillary, the nature and concentration of the electrolytic solution, the distance between the galvanometer and the photographic paper all remain constant. The practical determination of nitrate or nitrite is much simplified by first making calibration curves.

The necessary conditions for the determination of nitrates and nitrites being cleared up, the practical application of this method is carried out. If nitrates are determined from solutions not containing any components which influence the nitrate reduction, the sensitivity of this method is very large. For example, using a galvanometer of the sensitivity of $2,2 \cdot 10^{-8}$ amp/mm/m, 0.1 nm of the wave height corresponds to $3 \cdot 10^{-7}$ n. of nitrate ions with its 1/2 sensitivity, and, furthermore, the wave grows proportionally with the concentration of nitrates. If the solution contains some components which influence the nitrate reduction, the sensitivity of this method is lowered. Many determinations of nitrates were carried out with a sensitivity of 1/100 (full sensitivity: $7 \cdot 10^{-9}$ amp/mm/m) up to till 10^{-5} n exactly and rapidly.

The separation of nitrate and nitrite can be achieved by using the reduction of nitrate and nitrite in lanthanum chloride solution and the instability of nitrite in HCl solution. This procedure has been proved to be satisfactory. The determination of nitrates in the presence of sulphates can be made after the precipitation of sulphate ions with barium chloride and by filtration of the precipitates. According to the results of this experiment, however, the accuracy of this method is somewhat hampered, i. e. the error is within the range of $\pm 4\%$. Phosphate and organic acids such as oxalic, citric and similar acids should be removed from the solution, with which the nitrate determination is to be made.

The determination of nitrate or nitrite in the presence of bromates or iodates has also been studied. If traces of bromate or iodate are present in the solution and the nitrate exists in excess, this method can be used, but in the reverse case it can not be carried out quantitatively.

As a practical application and proof of the suitability of the polarographic determination of nitrates, several samples of fertilisers containing nitrogen in the form of nitrate were analysed. They contained one or several kinds of the following ions: Ca^{++} , K^+ , NH_4^+ , PO_4''' . The results were compared with the volumetric method of Devarda. The polarographic method gave always value slightly lower than that obtained by the Devarda method, except in the case of the presence of PO_4''' . The greatest advantage of the polarographic method is the rapidity with which the determinations can be made. The Devarda method takes about two hours for one analysis, while the polarographic method, from the weighing of the sample to the developing of the photographic paper is accomplished in 20–25 minutes. This includes the taking of three curves at least. The presence of sulphate or phosphate prolongs the procedure by the time required for the precipitation. Compared with the determination of nitrates on stable electrodes, the polarographic method is more suitable owing to its accuracy and rapidity.

In conclusion from the results of these experiments, it may be

asserted, that the polarographic determination of nitrates and nitrites can find many applications in analytical chemistry. The most prominent characteristics of the polarographic method for the determination of nitrates and nitrites is the great sensitivity, which due to the large numbers of faraday for the complete reduction of one equivalent ion. Simplicity and rapidity are also excellent characteristics by this method. For practical purposes the first thing to be done is to make a calibration curve. When we have a calibration curve for a special case, the determination can be much simplified.

In general, for the determination of nitrates and nitrites in solutions, where no disturbing components affecting their waves are present no other method perhaps, can compete with the polarographic method as regards accuracy and simplicity. The presence of such ions lessens the excellence of this method. Of such ions anions are more troublesome than cations. Cations, which are reduced more negatively than nitrates and nitrites, present no difficulties. The detection and the removal of those cations, which are reduced more positively than nitrates and nitrites, may not be very difficult. The detection can be done simply by means of polarographs. As the non reducible and high valency anions cause trouble, before the application of the polarographic determination of nitrates and nitrites in unknown samples, the presence and the influence of such anions upon nitrate waves must necessarily be investigated. Such investigations had already been partially made by the present author. If such a problem in a new case could be solved and the calibration curves could be based upon this investigation, the polarographic method might be applied without great difficulty.

According to characteristics of this method, its suitability will be proved especially in the following two cases. 1) where a number of the determination of nitrates and nitrites in the almost same materials is carried out repeatedly, and the kinds and the concentration of components, besides nitrates and nitrites, always remain almost the same. In this case the influence of other components upon the nitrate or nitrite waves can be preliminarily investigated, and an

adequate calibration curve for the polarographic determination could be made upon this results. This example utilizes the simplicity and the rapidity of the polarographic method. Such examples are not few in the field of agricultural and industrial chemistry. 2) where the determination of nitrates or nitrites of a very small concentration is necessary, and other analytical methods can not be used for this purpose. Such a determination, of course, would not be an easy task even by the polarographic method too, but its extraordinary sensitivity for nitrates and nitrites and the accuracy would overcome this difficulty easier than other methods. This example is the utilization of the high sensitivity of the polarographic method for nitrate and nitrite ions.

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ENZYME CHEMICAL INVESTIGATION OF FORMOSAN SNAKE VENOMS, IV.*

STUDIES ON THE ACTIVATION OF PEPTIDASE BY THE SNAKE VENOMS

PART IV.

On the Activation Power of the Venom of Different Species of Formosan Snakes upon the Enzymatic Splitting of Alanylglycine and Leucylglycine.

(With 2 Text-Figures)

Yoshio TSUCHIYA

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INTRODUCTION

In a previous paper of this series,⁴⁾ the author reported that there had been found two different relations according to enzyme materials used when test was made on the activation power of the venom of Taiwanhabu upon the splitting of alanylglycine and leucylglycine.

Namely, in the case of using pig's kidney or tortoise's liver as the enzyme material, the activation power of the venom was marked upon the splitting of leucylglycine while not upon that of alanylglycine (Relation a); in the case of using pig's pancreas, no perceptible effect of the venom was observed upon the splitting of both leucylglycine and alanylglycine (Relation b).

Now there occurs a question how these relations result when various venoms of different species of snakes are used. In order to make this question clear, similar test was undertaken with the venoms of five kinds of Formosan snakes:—Taiwanhabu (*Trimeresurus mucrosquamatus*), Aohabu (*Trimeresurus gramineus*), Hyappoda (*Agkistrodon acutus*), Taiwankobura (*Naja naja atra*), and Amagasahebi (*Bungarus multicinctus*).

The experimental results thus obtained are summarized on p. 281.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO, for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI, for his assistance in the collection of snake venoms, etc. in the present work.

EXPERIMENTAL PART

A. Preparations Employed.

1. Substrate buffer solutions.

Substrates and substrate buffer solutions were prepared as noted previously.³⁾

2. Enzyme solutions.

a. Original enzyme materials and enzyme extracts.

Dried powder of each enzyme material was prepared in the same way as that previously noted.^{3,4)}

In table I is contained a survey of the enzyme materials and enzyme extracts employed, together with the particular data relating to their preparations.

b. Purified enzyme solutions. (eludates)

Each original enzyme extract was subjected to the adsorption with aluminium hydroxide $C\gamma^*$ at pH 5.0 and the successive elution from the adsorbate was made with dilute ammonia solution.

The eludate thus obtained was used as enzyme solution.

Details with regard to their preparations are given in each table concerned. (cf. the process on p. 181 in a previous paper⁵⁾)

3. Venom solutions.

a. Venomous snakes.

Five species of Formosan venomous snakes were collected.

i. Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)]

TABLE I.

Survey of the enzyme materials and enzyme extracts employed.

Enzyme materials				Enzyme extract				
No of material	Kinds of materials	Date of preparation	Water content %	No of extract	Date of preparation	g of material used	Total volume of mixture cc	Conc. of solvent G %
1	Dried liver of tortoise*	11 9 1934	9.0	I	25 6 1935	2	50	30
2	Dried kidney of pig	11/7 1935	10.6	IIa	26/8 1935	2	50	30
				IIb	4/9 1935	2	50	30
3	Dried pancreas of pig	11 7 1935	12.3	IIIa	20/8 1935	4	50	30
				IIIb	26 8 1935	4	50	30
				IIIc	4/9 1935	4	50	30

* This material was the same (No. 2) as that employed in a previous paper.²⁾

* This adsorption agent was the same as employed in a previous paper,⁷⁾ i. e., it contained 46.1 mg. Al₂O₃ per 10 cc and 30 % glycerine.

- ii. Aohabu [*Trimeresurus gramineus* (SHAW)]
- iii. Hyappoda [*Agkistrodon acutus* (GÜNTHER)]
- iv. Taiwankobura [*Naja naja atra* (CANTOR)]
- v. Amagasahebi [*Bungarus multicinctus* BLYTH]

b. *Snake venoms.*

i. *Fresh venoms.*

The fresh venoms were collected in the same manner as that previously permitted.²¹

The venoms in fresh state were a somewhat viscid fluid having an appearance like saliva, the tint of which varied from colorless to bright yellow according to the species of snakes; viz. Hyappoda, colorless; Taiwankobura and Amagasahebi; very slight yellow (almost colorless); Taiwanhabu and Aohabu, bright yellow.

The venoms of Taiwanhabu, Aohabu, and Hyappoda contained a few floating granular particles of whitish tint, which soon settled to the bottom of a glass vessel. (the receptacle)

The fresh venom contained usually from 65 to 87% of water.

The main part of each venom was readily soluble in water or dilute glycerine solution, but scarcely soluble in alcohol or ether.

ii. *Dried venoms.*

The dried venoms were prepared as usual by drying at room temperature as quickly as possible under suction in a vacuum desiccator over calcium chloride. The dried venom thus obtained presented similar appearance to that of an aggregation of crystals.

When the venom was mixed with sufficient quantity of water, there was obtained a solution which gave a slight opalescent color, and produced, on boiling, dense coagulum (Taiwanhabu and Taiwankobura), white turbidity (Hyappoda), or strong opalescent color (Aohabu and Amagasahebi).

Biulet, MILLON's, xantho-protein reactions were strongly positive.

c. *Venom solutions.*

Fine powder of each dried venom which had been prepared by crushing the aggregate in an agate mortar, was dissolved in 30% glycerine solution, centrifuged and filtered.

0.1 or 1% venom solution thus prepared was used in the experiment concerned.

B. Experimental Methods.

The experiments reported in the following were carried out in exactly the same way as that described previously.³

The determination of enzyme activity was made according to the semi-micro alcohol titration method as devised and modified by LINDERSTRÖM-LANG and SATO.¹

The digestive conditions were kept as follows, unless otherwise duly noted :

Substrate concentration=0.1 mol.,

Glycerine concentration=15 %,

pH=8.0±0.05 (ammonia-ammonium chloride buffer),

Digestion at 40°.

In all experiments using venom, each venom solution which had been mixed with the substrate buffer solution, was mixed with the enzyme solution precisely at the beginning of the digestion.

With regard to the digestion process, reference should be made to p. 141 in a previous paper.³

C. Symbols.

The symbols used in the present investigation were the same as those used in previous papers.^{3,4}

D. Experimental Results.

Experimental results obtained are given in tables IV~XII and illustrated in figures I and II. In table III, the author made a general survey of these results.

Important facts which were made clear by these results are summarized in the following section.

SUMMARY

1. With various venoms of different species of Formosan snakes, test was made on their activation powers upon the splitting of

alanylglycine and leucylglycine, by the dipeptidase of each adsorption eludate which was obtained from the dried powder of tortoise's liver, pig's kidney or pig's pancreas.

2. As clearly shown by table II, according to the combination of one of the venoms of different species of snakes and one of the enzyme materials, the author could find the following three different cases in the relations of behaviour of the venoms upon the splitting of alanylglycine and leucylglycine.

Relation a:—The activation power of the venom is slight or absent upon the splitting of alanylglycine while marked upon the splitting of leucylglycine.

Relation b:—The activation power of the venom is slight or absent upon the splitting of both alanylglycine and leucylglycine.

Relation c:—The activation power of the venom is marked upon the splitting of alanylglycine while slight or absent upon the splitting of leucylglycine.

TABLE II.

Relation of the behaviour of the venoms upon the splitting of alanylglycine and leucylglycine.

Venoms of different species of snakes and various enzyme materials are as shown in the following table. (cf. table III)

Kinds of snake venoms	Activation power of snake venoms			
	Kidney of pig or liver of tortoise		Pancreas of pig	
	AG	LG	AG	LG
Taiwanhabu	Slight or absent	Marked	Slight or absent	Slight or absent
Aohabu				
Hyappoda				
Taiwankobura	Slight or absent	Marked	Marked	Slight or absent
Amagasahebi	Slight or absent	Slight or absent	Marked	Slight or absent

Among these relations, (a) and (b) have already been found in a previous paper⁴ while (c) is a noticeable fact which was discovered for the first time in the present investigation.

3. Observing from the viewpoint of the relations (a, b, c) explained, the venoms of different species of snakes tested could be classified into the following three groups, i. e.,

First group: Taiwanhabu, Aohabu, and Hyappoda.

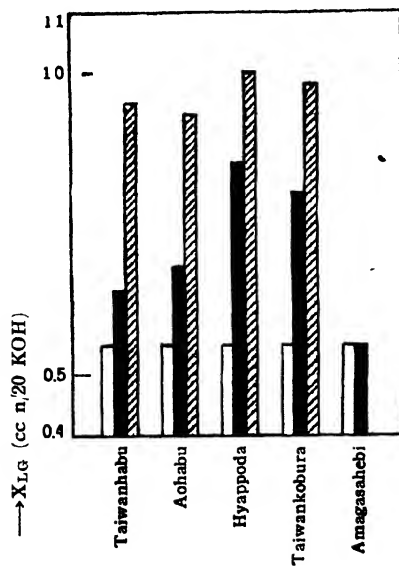
Second group: Taiwankobura.

Third group: Amagasahebi.

FIG. I.

Figures illustrating the activation power of various kinds of snake venoms upon the cleavage of LG by dipeptidase of the adsorption eludate obtained from the dried liver of tortoise.

The data correspond to the figures in table V.



→ Kinds of snake venoms,

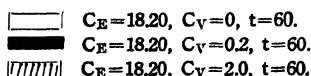
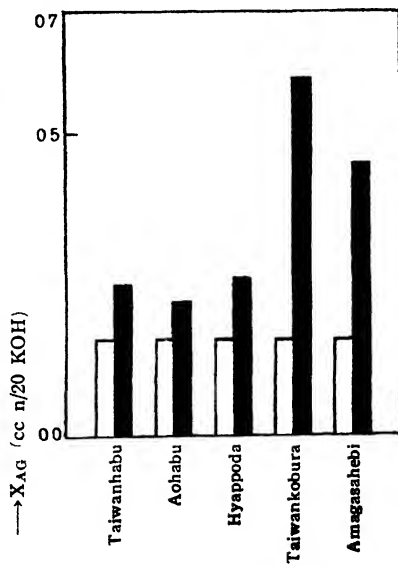


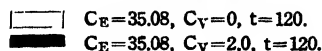
FIG. II.

Figures illustrating the activation power of various kinds of snake venoms upon the cleavage of AG by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

The data correspond to the figures in table VII.



→ Kinds of snake venoms.



Observing from the same viewpoint, a marked difference was also observed between the eludate (dipeptidase) of pig's kidney or tortoise's liver and that of pig's pancreas.

4. The venoms of different species of snakes were compared from the standpoint of the intensity of their activation powers a) upon the splitting of leucylglycine by the dipeptidase of tortoise's liver, and b) upon the splitting of alanylglycine by the dipeptidase of pig's pancreas and found to be in the following order:

In the case of a)

Hyappoda > Taiwankobura > Aohabu > Taiwanhabu > Amagasahebi

In the case of b)

Taiwankobura > Amagasahebi > Hyappoda > Aohabu > Taiwanhabu

TABLE III.

Activation power of the venoms of different species of snakes upon the cleavage of alanylglycine and leucylglycine by dipeptidase of the adsorption eludates obtained from various kinds of enzyme materials.

Kinds of snake venoms	Kinds of enzyme materials Peptides No.	Dried liver of tortoise			Dried kidney of pig		Dried pancreas of pig		
		AG		LG	AG		AG		LG
		1	2	3	4	5	6	7	8
Taiwanhabu		-	+	++	(-)	(+)	-		-
			(4th)				(5th)		
Aohabu		-	+	++	-	+	+	+	-
			(3rd)				(4th)		
Hyappoda		-	+	++	-	++	+	+	-
			(1st)				(3rd)		
Taiwankobura		-	+	++	(-)	(+++)	+++++		-(+)
			(2nd)				(1st)		
Amagasahebi		-	-		-	-	+++	+++	+
			(5th)				(2nd)		

Note: Columns 1, 2, and 3 correspond to the figures in table VI; 4 and 5 correspond to the figures in table X; 6 corresponds to the figures in table VIII; 7 and 8 correspond to the figures in table XII.

Numbers in parentheses denote the order of activation power of the venoms. (cf. table VI and VIII)

Concerning (-), etc., compare the results in 5th report. (p. 299).

TABLE IV.

The LG- and AG-cleavages by dipeptidase of various kinds of snake venoms. At the determination, $C_V=2.0$. Time of digestion=60 mins. for X_{LG} , and 120 mins for X_{AG} .

Kinds of snake venoms	$_X^*$	
	LG	AG
Taiwanhabu	0.02	0.06
Aohabu	0.01	0.02
Hyappoda	0.01	0.04
Taiwankobura	0.00	0.05
Amagasahebi	0.02	0.07

* Symbol $_X$ as noted on p. 171 in a previous paper.⁴⁾ The values of $_X$ are to be taken for the calculation of A_{AG} or A_{LG} , per cent of activation in the following tables.

TABLE V.

Activation power of various kinds of snake venoms upon the cleavage of leucyl-glycine and alanylglycine by dipeptidase of the adsorption eluate obtained from the dried liver of tortoise.

20cc of enzyme extract I+8.0cc of n/100 acetic acid + 4.0cc of Al OH $C_V+8.0$ cc of 30% glycerine solution \rightarrow 40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the adsorbate were added 19.2 cc of n 100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 20 cc of the eluate were added 10.8 cc of n 100 acetic acid and 9.2 cc of 30% glycerine solution total volume 40 cc. The eluate of pH 5.9 =pH of the original enzyme extract thus obtained was used.

At the determination, $C_F=18.20$ for X_{LG} , and 3.64 for X_{AG} . Time of digestion=60 mins.

Kinds of snake venoms	X_{LG}			X_{AG}	
	$C_V=0$	$C_V=0.2$	$C_V=2.0$	$C_V=0$	$C_V=2.0$
Taiwanhabu	0.54	0.64 0.65*	0.95	1.05	1.09
Aohabu	0.54	0.68	0.93	1.05	1.04
Hyappoda	0.54	0.85	1.00	1.05	1.05
Taiwankobura	0.54	0.80	0.98	1.05	1.08
Amagasahebi	0.54	0.54	—	1.05	1.03

* The venom collected during hibernation was used.

TABLE VI.

Calculation of the activation power of various kinds of snake venoms upon the LG- and AG-cleavages by dipeptidase of the adsorption eludate obtained from the dried liver of tortoise.

The data correspond to the figures in table V.

Kinds of snake venoms	Activation power						
	LG					AG	
	Per cent of activation $A_{LG}^{*2)}$		Relative rate of activation ^{*3)}		Order of activation	Per cent of activation $A_{AG}^{*2)}$	Relative rate of activation ^{*3)}
	$C_V=0.2$	$C_V=2.0$	$C_V=0.2$	$C_V=2.0$		$C_V=2.0$	$C_V=2.0$
Taiwanhabu	+19 +20 ^{*1)}	+72	+	++	4th	+1	—
Aohabu	+26	+70	+	++	3rd	-2	—
Hyappoda	+57	+83	+	++	1st	-1	—
Taiwankobura	+48	+81	+	++	2nd	0	—
Amagasahebi	0		—		5th	-1	—

*1) The venom collected during hibernation was used.

*2) A_{LG} , A_{AG} , etc. were calculated as noted on p. 169 in a previous paper.¹⁾ (cf. ΔX on Table IV, p. 285)

*3) Relative rate of activation +, ++, —, etc. as noted on p. 171 in a previous paper.⁴⁾

TABLE VII.

Activation power of various kinds of snake venoms upon the AG-cleavage by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

20 cc of enzyme extract IIIa+2.1 cc of n/10 acetic acid+16.0 cc of $Al(OH)_3$ C_V +1.9 cc of 30%glycerine solution→40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the adsorbate were added 16.8 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eludate were added 1.8 cc of n/100 acetic acid and 8.2 cc of 30% glycerine solution (total volume 20 cc). The eludate of pH 5.9 (=pH of the original enzyme extract) thus obtained was used.

At the determination, $C_R=35.08$. Time of digestion=120 mins.

Kinds of snake venoms	X_{AG}	
	$C_V=0$	$C_V=2.0$
Taiwanhabu	0.16	0.25
Aohabu	0.16	0.22
Hyappoda	0.16	0.26
Taiwankobura	0.16	0.59
Amagasahebi	0.16	0.45

TABLE VIII.

Calculation of the activation power of various kinds of snake venoms upon the AG-cleavage by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

The data correspond to the figures in table VII.

Kinds of snake venoms	Activation power		
	Per cent of activation A_{AG}	Relative rate of activation	Order of activation
Taiwanhabu	+19	—	5th
Aohabu	+25	+	4th
Hyappoda	+33	+	3rd
Taiwankobura	+238	+++++	1st
Amagasahebi	+138	+++	2nd

Note: cf. foot note on table VI, p. 286.

TABLE IX.

Activation power of snake venoms upon the cleavages of AG and LG by dipeptidase of the adsorption eludate obtained from the dried kidney of pig.

20 cc of enzyme extract Ila or I Ib + 10.46 cc of n 100 acetic acid + 4.0 cc of $Al(OH)_3$ + 5.54 cc of 30% glycerine solution \rightarrow 40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the adsorbate were added 19.2 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 15 cc of the eludate were added 7.5 cc of n/100 acetic acid and 7.5 cc of 30% glycerine solution (total volume 30 cc). Thus the eludate of pH 6.2 (=pH of the original enzyme extract) was obtained. For the venoms of Aohabu and Hyappoda, test was made with the eludate which was prepared from Ila, and for the venom of Amagasahebi with that of I Ib.

At the determination, $C_E=17.74$. t=time of digestion, mins.

Kinds of snake venoms	X					
	AG			LG		
	t	$C_V=0$	$C_V=2.0$	t	$C_V=0$	$C_V=2.0$
Aohabu	15	0.75	0.80	60	0.44	0.63
Hyappoda	15	0.75	0.83	60	0.44	0.78
Amagasahebi	15	0.60	0.64	60	0.32	0.35

TABLE X.

Calculation of the activation power of snake venoms upon the cleavages of LG and AG by dipeptidase of the adsorption eludate obtained from the dried kidney of pig

The data correspond to the figures in table IX.

Kinds of snake venoms	Activation power			
	AG		LG	
	Per cent of activation A_{AG}	Relative rate of activation	Per cent of activation A_{LG}	Relative rate of activation
Aohabu	+5	—	+41	+
Hyappoda	+9	—	+75	++
Amagasahebi	+3	—	+6	—

Note: cf. foot note on table VI, p. 283.

TABLE XI.

Activation power of snake venoms upon the cleavages of AG and LG by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

20 cc of enzyme extract IIIb or IIIc + 2.1 cc of n 10 acetic acid + 16.0 cc of Al OH), C_V + 1.9 cc of 30% glycerine solution \rightarrow 40 cc pH 5.0, centrifuged. The residual solution was discarded. To the adsorbate were added 16.8 cc of n/100 ammonia solution (total volume 20 cc) and shaken well.

After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eludate were added 1.8 cc of n/100 acetic acid and 8.2 cc of 30% glycerine solution (total volume 20 cc). Thus the eludate of pH 5.9 (pH of the original enzyme extract) was obtained. For the venoms of Aohabu and Hyappoda, test was made with the eludate which was prepared from IIIb, and for the venom of Amagasahebi with that of IIIc.

At the determination, C_E = 35.03, t = time of digestion, mins.

Kinds of snake
venoms

	AG			LG		
	t	$C_V=0$	$C_V=2.0$	t	$C_V=0$	$C_V=2.0$
Aohabu	240	0.20	0.28	60	0.32	0.34
Hyappoda	240	0.20	0.29	60	0.32	0.37
Amagasahebi	240	0.19	0.51	60	0.42	0.71

TABLE XII.

Calculation of the activation power of snake venoms upon the cleavages of AG and LG by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

The data correspond to the figures in table XI.

Kinds of snake venoms	Activation power			
	AG		LG	
	Per cent of activation $A_{A(1)}$	Relative rate of activation	Per cent of activation $A_{L(1)}$	Relative rate of activation
Aohabu	+30	+	+3	—
Hyappoda	+25	+	+3	—
Amagasahebi	+142	+++	+52	†

Note: cf. foot note on table VI, p. 286.

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PART V.

On the Activation Power of the Venom of *Taiwankobura* [*Naja naja atra* (CANTOR)] upon the Enzymatic Splitting of Various Kinds of Dipeptides

(With 7 Text-Figures)

Yoshio TSUCHIYA

(Accepted for publication, April 4, 1936)

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INTRODUCTION

In previous papers,^{5,6,7,8)} the activation power of the snake venoms was tested mainly upon the enzymatic splitting of leucylglycine (=LG) and alanylglycine (=AG). Therefore, in the present investigation, the author studied the activation power of the venom of

Taiwankobura* (*Naja naja atra*) upon the enzymatic splitting of various kinds of dipeptides such as leucylglycine (=LG), glycylleucine (=GL), glycyphenylalanine (=GPh), glycyvaline (=GV), alanylglycine (=AG), valylglycine (=VG), and glycyglycine (=GG).

As the enzyme solution, was used each adsorption eludate which was obtained from the dried powder of the liver of tortoise or from those of intestinal mucous membrane, kidney and pancreas of pig. The experimental results obtained are summarized on p. 295.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO., for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI., for the assistance he has given to this work.

EXPERIMENTAL PART

A. Preparations Employed.

1. *Substrate-buffer solutions.*

a. *Substrates.*

Dipeptides employed as substrates were all racemic.

LG, GL, and GPh were prepared according to FISCHER's method,¹⁾ GV and VG according to the method of LEVENE and his co-workers,³⁾ and GG according to the method of SATO and the author⁴ or according to that of FREUDENBERG and his co-workers.²

AG was first synthesized by E. FISCHER¹⁾ by heating a mixture of 1 part of α -bromopropionylglycine and 5 parts of ammonium hydroxide (25 %) in a sealed tube at 100° for 20 minutes.

In the present case, this procedure was not followed.

Instead, the amination of α -bromopropionylglycine was directly carried out owing to this halogen-compound being very unstable, by allowing the reaction mixture which was obtained by the combination of glycocoll and α -bromopropionylbromide in the presence of

* The venom was selected owing to its high activation power upon the splitting of LG and AG by the dipeptidase of the enzyme materials tested.

alkali (NaOH) to stand for 3 days at room temperature in 25 % ammonium hydroxide concentration.

The resulting mixture was then evaporated to dryness on the water-bath and treated with absolute methyl alcohol.

The peptide thus obtained was recrystallized from 50 % hot alcohol.

b. Substrate-buffer solutions.

The substrate-buffer solutions were prepared as previously noted.⁵⁾

2. Enzyme solutions.

a. Original enzyme materials and enzyme extracts.

These were prepared in exactly the same way as that previously described.⁵⁾

Table I contains a survey of the enzyme materials and enzyme extracts employed, together with the particular data relating to their preparations.

TABLE I.
Survey of the enzyme materials and enzyme extracts employed

Enzyme material				Enzyme extract				
No. of material	Kinds of materials	Date of preparation	Water content %	No. of extract	Date of preparation	g of material used	Total volume of mixture cc	Conc. of solvent G %
1	Dried kidney of pig	8/2 1935	8.8	Ia	28/6 1935	2	50	30
2	"	11/7 1935	10.6	Ib	27/7 1935	2	50	30
3	Dried intestinal mucous membrane of pig	10/2 1935	9.0	II	16/7 1935	2	50	30
4	Dried liver of tortoise	11/9 1934	9.0	III	10/7 1935	2	50	30
5	Dried pancreas of pig	10/2 1935	10.9	IVa	9/7 1935	2	25	30
	"	31/5 1935	21.7	IVb	6/7 1935	4	50	30
	"	11/7 1935	12.3	IVc	28/7 1935	4	50	30

b. Enzyme solutions. (eludates)

Each enzyme solution was prepared from each original enzyme extract, by the adsorption with aluminium hydroxide C_7^* and the successive elution from the adsorbate with dilute ammonia solution.

Details with regard to their preparations are given respectively in each table concerned. (cf. the process on p. 181 in a previous paper⁷⁾).

3. Venom solutions.

Dried venom of Taiwankobura [*Naja naja atra* (CANTOR)] was prepared in the same way as before, and 0.5 or 1 % glycerine (30 %) solution of the venom was newly prepared just before use.

B. Experimental Methods.

Experiments were carried out in exactly the same way as previously described.⁸⁾

C. Symbols.

Symbols used were the same as those described in previous papers.^{5,6)}

D. Experimental Results.

Results obtained are given in tables II~XVIII and illustrated in corresponding figures I~IV.

Since the important facts discovered are summarized in the following section, no further detailed comment will be necessary here except that, for comparison of the activation powers of the venom upon the splitting of various dipeptides, were adopted the values under the same experimental conditions.

* This adsorption agent was the same as employed in a previous paper⁷⁾ i. e., it contained 46.1 mg Al_2O_3 per 10 cc and 30% glycerine.

SUMMARY

1. In the present experiment, test was made on the activation power of the venom of *Taiwankobura* upon the enzymatic splitting of various kinds of dipeptides such as LG, GL, GPh, GV, AG, VG, and GG. As the enzyme solution was used each adsorption eluate which was obtained from the dried powder of the liver of tortoise or from those of intestinal mucous membrane, kidney, and pancreas of pig.

Table II gives a survey of the present experimental results, according to which, the following facts can be pointed out :

2. In the case of using, as enzyme material, the intestinal mucous membrane or kidney of pig, marked activation power of the venom was observed upon the splitting of LG, GL, and GPh, (VG), while it was slight or absent upon the splitting of AG, VG, and GG. A similar behaviour of the venom upon the splitting of LG and AG was also noticed in the case when another enzyme sample of the pig's kidney was employed. In the case of using the liver of tortoise, a similar result was also obtained except that the activation power upon the splitting of LG was less marked.

3. In the case of using, as enzyme material, the pancreas of pig, a remarkable fact was found that the splitting of AG was activated very markedly by the venom—a fact which had never been found in the case of using other enzyme materials.

As for other peptides, the activation power of the venom was marked upon the splitting of GL and GPh while it was far less marked upon the splitting of LG and GG, and it was absent upon the splitting of VG.

Similar behaviour of the venom upon the splitting of AG and LG was also noticed in the case when two other different samples of the pig's pancreas were employed.

4. The eluate (dipeptidase solution) of the pancreas of pig can be clearly distinguished from the eluate of other enzyme materials such as the kidney or the intestinal mucous membrane of pig or

the liver of tortoise, from the viewpoint that the activation power of the venom upon the splitting of AG is very marked in the former case while it is practically absent in the latter cases.

It should also be noticed here that the activation power of the venom upon the splitting of LG is far less marked (or absent) in the former case as compared with that in the latter cases.

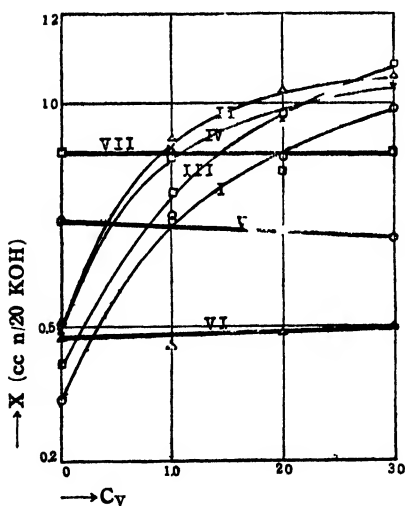
6. Specific nature of the eludate of the pancreas of pig which is different from the group of other enzyme materials above stated was also observed from the viewpoint of the ratio of each splitting of various kinds of dipeptides, i. e., the data shown by table III

FIG. 1.

Curves illustrating the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried kidney of pig.

A. The curves correspond to the figures in table V.

A.

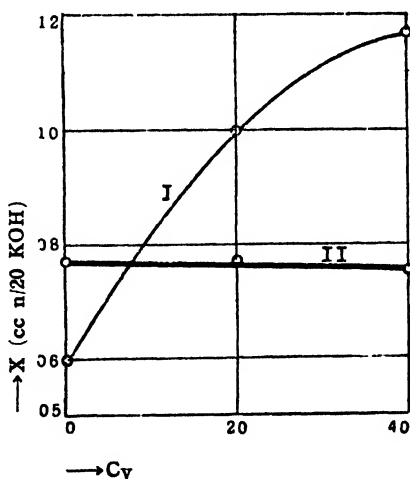


Curves:

- I, Leucylglycine (LG), $t=60$
 - II, Glycylleucine (GL), $t=30$
 - III, Glycylphenylalanine (GPh), $t=60$
 - IV, Glycylvaline (GV), $t=30$
 - V, Alanylglycine (AG), $t=15$
 - VI, Valylglycine (VG), $t=30$
 - VII, Glycylglycine (GG), $t=240$
- } $C_E = 14.59$

B. The curves correspond to the figures in table VII.

B.



Curves:

- I, Leucylglycine (LG), $t=60$
 $C_E = 17.74$
- II, Alanylglycine (AG), $t=10$
 $C_E = 17.74$

FIG. II.

Curves illustrating the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried intestinal mucous membrane of pig.

The curves correspond to the figures in table IX.

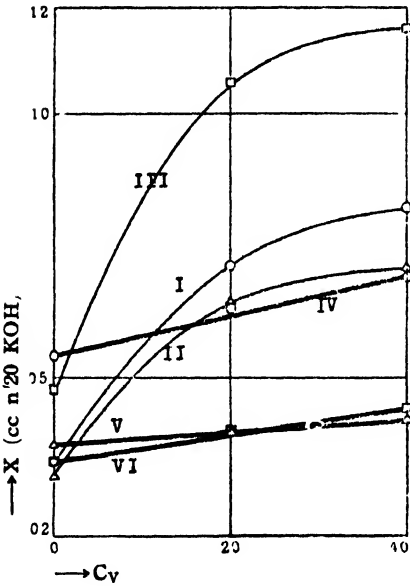


Fig. II. Curves: I, Leucylglycine (LG), $t=120$
II, Glycylleucine (GL), $t=30$
III, Glycylphenylalanine (GPh), $t=120$
IV, Alanylglycine (AG), $t=15$
V, Valylglycine (VG), $t=30$
VI, Glycylglycine (GG), $t=120$ } $C_E=18.20$.

FIG. III.

Curves illustrating the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried liver of tortoise.

The curves correspond to the figures in table XI.

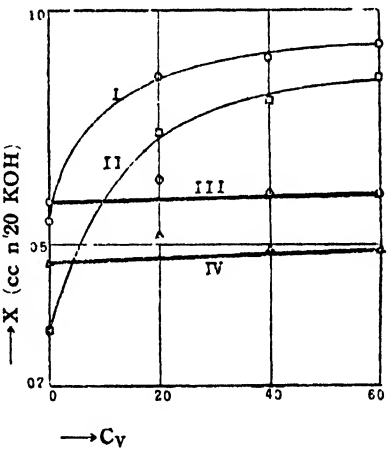


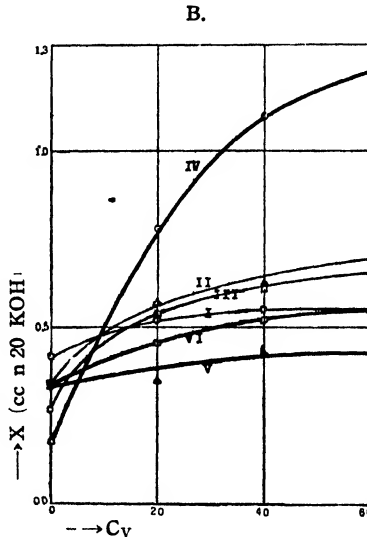
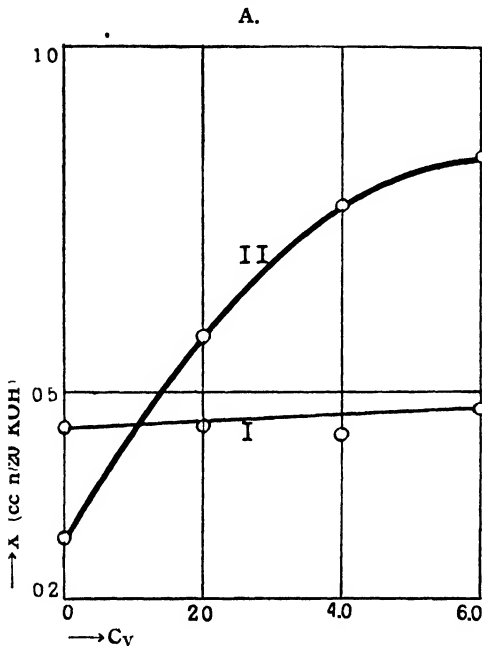
Fig. III. Curves: I, Leucylglycine (LG), $t=60$
II, Glycylphenylalanine (GPh), $t=30$
III, Alanylglycine (AG), $t=7.5$
IV, Valylglycine (VG), $t=15$ } $C_E=18.20$.

FIG. IV.

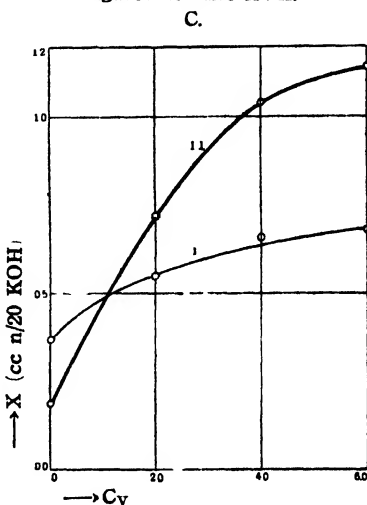
Curves illustrating the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

A. The curves correspond to the figures in table XIII.

B. The curves correspond to the figures in table XV.



C. The curves correspond to the figures in table XVII.



A. Curves:

I, Leucylglycine (LG), $t=60$
II, Alanyl glycine (AG), $t=240$ } $C_E=35.64$.

B. Curves:

I, Leucylglycine (LG), $t=60$
II, Glycylleucine (GL), $t=240$
III, Glycylphenyl-
alanine (GPh), $t=240$ } $C_E=31.32$.
IV, Alanyl glycine (AG), $t=300$
V, Valyl glycine (AG), $t=300$
VI, Glycylglycine (GG), $t=300$

C. Curves:

I, Leucylglycine (LG), $t=60$
II, Alanyl glycine (AG), $t=120$ } $C_E=35.08$.

TABLE II.

Activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludates obtained from various kinds of enzyme materials.

The data correspond to the figures in tables VI, VIII, X, XII, XIV, XVI and XVIII, with special reference to the case when the activation was tested with $C_V=2.0$.

Substrate (Symbol)	Kinds of enzyme materials						
	Dried kidney of pig		Dried in- testinal mucous membrane of pig	Dried liver of tor- toise	Dried pancreas of pig		
	1	2	3	4	5	6	7
Leucylglycine (LG)	++++	++	+++	+	-	+	+
Glycylleucine (GL)	+++		+++			++	
Glycylphenyl- alanine (GPh)	+++		+++	+++		+++	
Glycylvaline (GV)	++						
Alanylglycine (AG)	-	-	-	-	++	+++++	+++++
Valylglycine (VG)	-		-	-		-	
Glycylglycine (GG)	-		-			+	

Columns: 1 corresponds to the figures in table VI (cf. Fig. I A);
 2 corresponds to the figures in table VIII (cf. Fig. I B);
 3 corresponds to the figures in table X (cf. Fig. II);
 4 corresponds to the figures in table XII (cf. Fig. III);
 5 corresponds to the figures in table XIV (cf. Fig. IV A);
 6 corresponds to the figures in table XVI (cf. Fig. IV B);
 7 corresponds to the figures in table XVIII (cf. Fig. IV C).

TABLE III.

Ratio of each cleavage of various kinds of peptides to that of leucylglycine by dipeptidase of the adsorption eludates obtained from various kinds of enzyme materials.

The data correspond to the figures in tables V, VII, IX, XI, XIII, XV, and XVII, with special reference to the case when the test was made with $C_V=0$.

$[X]/[X_{LG}]$, where $[X]$ or $[X_{LG}]=X$ or X_{LG} when $C_X=1$ and $t=60$ (as noted on p. 168 in a previous paper⁴⁾).

Enzyme solution		[X] / [X _{LG}]						
Kinds of materials	Extracts used for purification	LG	GL	GPh	AG	VG	GV	GG
Dried kidney of pig	Ia Ib	1 1	3.0	1.2	8.8 7.8	2.8	2.9	0.7
Dried intestinal mucous membrane of pig	II	1	3.6	1.4	12.7	4.4		1.0
Dried liver of tortoise	III	1		1.2	8.6	3.3		
Dried pancreas of pig	IVa IVb IVc	1 1 1	0.2	0.2	0.2 0.1 0.3	0.2		0.2

TABLE IV.

Cleavages of various kinds of peptides by dipeptidase of the venom of Taiwan-kobura.

At the determination, C_v -2.0. Time of digestion =120 mins.

Substrate (Symbol)	ΔX*	
Leucylglycine (LG)	0.00	* Symbol -X as noted on p. 171 in a previous paper. ¹⁾ The values of ΔX are to be taken for the calculation of A _{AG} , A _{LG} , etc. (per cent of activation) in the following tables.
Glycylleucine (GL)	0.00	
Glycylphenylalanine (GPh)	0.00	
Glycylvaline (GV)	0.01	
Alanylglycine (AG)	0.05	
Valylglycine (VG)	0.02	
Glycylglycine (GG)	0.00	

TABLE V.

Activation power of the venom of Taiwankobura upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried kidney of pig.

20 cc of enzyme extract Ia +5.4 cc of n/100 acetic acid +4.0 cc of Al(OH)₃, C_v +10.6 cc of 30% glycerine solution →40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 19.2 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged, and filtered. To 10 cc of the eludate were added 6.0 cc of n/100

acetic acid and 4.0 cc of 30% glycerine solution (total volume 20 cc). The eluate of pH 5.9 (=pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, $C_E=14.59$. t —time of digestion.

Substrate (symbol)	t mins.	X			
		$C_V=0$	$C_V=1.0$	$C_V=2.0$	$C_V=3.0$
Leucylglycine (LG)	60	0.34	0.74	0.88	0.99
Glycylleucine (GL)	30	0.51	0.92	1.03	1.06
Glycylphenylalanine (GPh)	60	0.42	0.80	0.98	1.09
Glycylvaline (GV)	30	0.50	0.90	0.97	1.04
Alanylglycine (AG)	15	0.74	0.75	0.71	0.70
Valylglycine (VG)	30	0.48	0.46	0.49	0.50
Glycylglycine (GG)	240	0.89	0.89	0.85	0.89

TABLE VI.

Calculation of the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried kidney of pig.

The data correspond to the figures in table V.

Substrate (Symbol)	Activation power					
	Per cent of activation $A^{*1)}$			Relative rate of activation $^{*2)}$		
	$C_V=1.0$	$C_V=2.0$	$C_V=3.0$	$C_V=1.0$	$C_V=2.0$	$C_V=3.0$
Leucylglycine (LG)	+118	+159	+191	+++	+++++	+++++
Glycylleucine (GL)	+80	+102	+108	++	+++	+++
Glycylphenylalanine (GPh)	+90	+133	+160	++	+++	+++++
Glycylvaline (GV)	+80	+94	+108	++	++	+++
Alanylglycine (AG)	+1	-5	-7	-	-	-
Valylglycine (VG)	-4	0	+2	-	-	-
Glycylglycine (GG)	0	-4	0	-	-	-

*1) A was calculated as noted on p. 169 in a previous paper;^(b) cf. ΔX on table IV, p. 300,

*2) Relative rate of activation +, ++, -, etc. as noted on p. 171 in a previous paper.^(b)

TABLE VII.

Activation power of the venom of Taiwankobura upon the cleavages of AG and LG by dipeptidase of the adsorption eluate obtained from the dried kidney of pig. 20 cc of enzyme extract lb+10.46 cc of n/100 acetic acid+4.0 cc of $Al(OH)_3$, C_V +5.54 cc of 30% glycerine solution \rightarrow 40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 19.2 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eluate were added 5.0 cc of n/100 acetic acid and 5.0 cc of 30% glycerine solution (total volume 20 cc). The eluate of pH 6.2 (=pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, $C_E=17.74$. t =time of digestion.

Substrate (symbol)	t mins.	X		
		$C_V=0$	$C_V=2.0$	$C_V=4.0$
Leucylglycine (LG)	60	0.60	1.00	1.17
Alanylglycine (AG)	10	0.77	0.77	0.75

TABLE VIII.

Calculation of the activation power of the venom of Taiwankobura upon the cleavages of AG and LG by dipeptidase of the adsorption eluate obtained from the dried kidney of pig.

The data correspond to the figures in table VII.

Substrate (symbol)	Activation power			
	Per cent of activation A		Relative rate of activation	
	$C_V=2.0$	$C_V=4.0$	$C_V=2.0$	$C_V=4.0$
Leucylglycine (LG)	+67	+94	++	++
Alanylglycine (AG)	0	-4	-	-

Note: cf. foot note on table VI, p. 301.

TABLE IX.

Activation power of the venom of Taiwankobura upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried intestinal mucous membrane of pig.

20 cc of enzyme extract II+10.0 cc of n/100 acetic acid+4.0 cc of $\text{Al}(\text{OH})_3$ C_Y+6.0 cc of 30% glycerine solution→40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 19.3 cc of n/100 ammonia solution (total volume 20 cc) and shaken well.

After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eluate were added 7.0 cc of n/100 acetic acid and 3.0 cc of 30% glycerine solution (total volume 20 cc). The eluate of pH 5.8 (=pH of the original enzyme extract) thus obtained was immediately used without standing.

At the determination, C_E=18.20. t=time of digestion.

Substrate (symbol)	t mins.	X		
		C _V =0	C _V =2.0	C _V =4.0
Leucylglycine (LG)	120	0.34	0.71	0.82
Glycylleucine (GL)	30	0.31	0.64	0.70
Glycylphenylalanine (GPh)	120	0.48	1.06	1.16
Alanylglycine (AG)	15	0.54	0.63	0.69
Valylglycine (VG)	30	0.37	0.40	0.42
Glycylglycine (GG)	120	0.34	0.40	0.44

TABLE X.

Calculation of the activation power of the venom of Taiwankobura upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried intestinal mucous membrane of pig.

The data correspond to the figures in table IX.

Substrate (symbol)	Activation power			
	Per cent of activation A		Relative rate of activation	
	C _V =2.0	C _V =4.0	C _V =2.0	C _V =4.0
Leucylglycine (LG)	+109	+141	+++	++++
Glycylleucine (GL)	+121	+142	+++	++++
Glycylphenylalanine (GPh)	+106	+126	+++	+++
Alanylglycine (AG)	+15	+26	—	+
Valylglycine (VG)	+5	+11	—	—
Glycylglycine (GG)	+18	+29	—	+

Note: cf. foot note on table VI, p. 301.

TABLE XI.

Activation power of the venom of Taiwankobura upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried liver of tortoise.

15 cc of enzyme extract III+6.0 cc of n/100 acetic acid +3.0 cc of $\text{Al}(\text{OH})_3$ Cy +6.0 cc of 30% glycerine solution \rightarrow 30 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 14.4 cc of n/100 ammonia solution (total volume 15 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eludate were added 5.4 cc of n/100 acetic acid and 4.6 cc of 30% glycerine solution (total volume 20 cc). The eludate of pH 6.0 (=pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, $C_E=18.20$. t =time of digestion.

Substrate (symbol)	t mins.	X			
		$C_V=0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	60	0.55	0.86	0.90	0.93
Glycylphenylalanine (GPh)	30	0.32	0.74	0.81	0.86
Alanylglycine (AG)	7.5	0.59	0.64	0.61	0.61
Valylglycine (VG)	15	0.46	0.52	0.49	0.49

TABLE XII.

Calculation of the activation power of the venom of Taiwankobura upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried liver of tortoise.

The data correspond to the figures in table XI.

Substrate (symbol)	Activation power					
	Per cent of activation A			Relative rate of activation		
	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	+56	+64	+69	+	++	++
Glycylleucine (GL)	+131	+153	+169	+++	++++	++++
Alanylglycine (AG)	+8	+2	+2	-	-	-
Glycylglycine (GG)	+13	+4	+4	-	-	-

* Note: cf. foot note on table VI, p. 301.

TABLE XIII.

Activation power of the venom of *Taiwankobura* upon the cleavages of AG and LG by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig. 10 cc of enzyme extract IVa+0.57 cc of n/10 acetic acid+8.0 cc of $\text{Al}(\text{OH})_3$ C_V +1.43 cc of 30% glycerine solution—20 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 8.4 cc of n/100 ammonia solution (total volume 10 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 5 cc of the eludate were added 2.3 cc of n/100 acetic acid and 2.7 cc of 30% glycerine solution (total volume 10 cc). The eludate of pH 5.6 (- pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, $C_T=35.64$. t =time of digestion.

Substrate (symbol)	t mins.	X			
		$C_V=0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	60	0.45	0.45	0.44	0.48
Alanylglycine (AG)	240	0.29	0.58	0.77	0.84

TABLE XIV.

Calculation of the activation power of the venom of *Taiwankobura* upon the cleavages of AG and LG by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

The data correspond to the figures in table XIII.

Substrate (symbol)	Activation power					
	Per cent of activation A			Relative rate of activation		
	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	0	-2	+7	-	-	-
Alanylglycine (AG)	+66	+97	+86	++	++	++

Note: cf. foot note on table VI, p. 301.

TABLE XV.

Activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eludate obtained from the dried pancreas of pig.

20 cc of enzyme extract IVb+2.9 cc of n/10 acetic acid +16.0 cc of $\text{Al}(\text{OH})_3$ C_V +1.1 cc of 30% glycerine solution—40 cc (pH 5.0), centrifuged. The residual

solution was discarded. To the residue were added 16.8 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eluate were added 3.6 cc of n/100 acetic acid and 6.4 cc of 30% glycerine solution (total volume 20 cc). The eluate of pH 6.2 (=pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, $C_E=31.32$. t =time of digestion.

Substrate (symbol)	t mins.	X			
		$C_V=0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	60	0.42	0.52	0.55	0.55
Glycylleucine (GL)	240	0.34	0.57	0.63	0.70
Glycylphenylalanine (GPh)	240	0.27	0.54	0.61	0.67
Alanylglycine (AG)	300	0.18	0.78	1.10	1.23
Valylglycine (VG)	300	0.33	0.35	0.43	0.45
Glycylglycine (GG)	300	0.34	0.46	0.52	0.55

TABLE XVI.

Calculation of the activation power of the venom of *Taiwankobura* upon the cleavages of various kinds of peptides by dipeptidase of the adsorption eluate obtained from the dried pancreas of pig.

The data correspond to the figures in table XV.

Substrate (symbol)	Activation power					
	Per cent of activation A			Relative rate of activation		
	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$	$C_V=2.0$	$C_V=4.0$	$C_V=6.0$
Leucylglycine (LG)	+24	+31	+31	+	+	+
Glycylleucine (GL)	+68	+85	+106	++	++	+++
Glycylphenylalanine (GPh)	+100	+126	+148	+++	+++	++++
Alanylglycine (AG)	+261	+372	+372	++++	++++	++++
Valylglycine (VG)	-9	0	-18	-	-	-
Glycylglycine (GG)	+35	+53	+62	+	+	++

Note: cf. foot note on table VI, p. 301.

TABLE XVII.

Activation power of the venom of *Taiwankobura* upon the cleavages of AG and LG by dipeptidase of the adsorption eluate obtained from the dried pancreas of pig 20 cc of enzyme extract IVc + 2.1 cc of n/10 acetic acid + 16.0 cc of Al(OH)₃ C₇ + 1.9 cc of 30% glycerine solution → 40 cc (pH 5.0), centrifuged. The residual solution was discarded. To the residue were added 16.8 cc of n/100 ammonia solution (total volume 20 cc) and shaken well. After ca. 30 mins' standing, centrifuged and filtered. To 10 cc of the eluate were added 1.8 cc of n/100 acetic acid and 8.2 cc of 30% glycerine solution (total volume 20 cc).

The eluate of pH 5.9 (pH of the original enzyme extract) thus obtained was immediately used without standing. At the determination, C_E=35.08. t= time of digestion

Substrate (symbol)	t mins.	X			
		C _V = 0	C _V - 2.0	C _V - 4.0	C _V - 6.0
Leucylglycine (LG)	60	0.37	0.55	0.66	0.68
Alanylglycine (AG)	120	0.19	0.92	1.04	1.14

TABLE XVIII.

Calculation of the activation power of the venom of *Taiwankobura* upon the cleavages of AG and LG by dipeptidase of the adsorption eluate obtained from the dried pancreas of pig.

The data correspond to the figures in table XVII.

Substrate (symbol)	Activation power					
	Per cent of activation A			Relative rate of activation		
	C _V =2.0	C _V =4.0	C _V =6.0	C _V =2.0	C _V =4.0	C _V =6.0
Leucylglycine (LG)	+49	+78	+84	+	++	++
Alanylglycine (AG)	+253	+395	+521	++++	++++	++++

Note: cf. foot note on table VI, p. 301.

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PART VI.

On the Adsorption and Elution of the Active Constituent of the Venom of Taiwankobura [*Naja naja atra* (CANTOR)]

(With 3 Text-Figures)

Yoshio TSUCHIYA

(Accepted for publication, April 4, 1936)

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INTRODUCTION

In previous papers,^{1 2 3 4 5)} specific activation power of the snake venoms upon dipeptidase was studied in detail with the original solution of the dried native venoms. In the present investigation,

the original solution of the dried native venom of Taiwankobura (*Naja naja atra*) was subjected to the adsorption with $\text{Al}(\text{OH})_3$ C γ and then to elution with n/100 acetic acid and it was observed how the active constituent (or active constituents) of the venom for the activation of dipeptidase in splitting of alanylglycine and leucylglycine pass over into the residual solution as well as into the eluate from the original venom solution.

The results obtained are summarized on p. 315.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO, for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI for the assistance he has given to the present work.

EXPERIMENTAL PART

A. Preparations Employed.

1. Substrate-buffer solutions.

AG and LG as substrates were prepared as noted in a previous paper,⁵⁾ substrate-buffer solutions as previously noted.¹⁾

2. Enzyme solutions.

As enzyme materials, were used dried kidney and pancreas of pig, from which each eluate was newly prepared just before use in such a way as given in the following examples:

a. The eluate of dried kidney of pig.

20 cc of original enzyme (30% G extract Ia were mixed with 10.46 cc of n/100 acetic acid (30% G) solution and 5.54 cc of 30% glycerine solution, and then subjected to the adsorption with the addition of 4.0 cc of $\text{Al}(\text{OH})_3$ C γ . As to the adsorption mixture, the pH was ca. 5.0, the glycerine conc. 30% and the total volume 40 cc i. e., twice the volume of original extract. The mixture was well shaken and left to stand for ca. 5 mins., centrifuged and filtered. The residual solution was discarded. The adsorbate was subjected to elution for ca. 30 mins. under occasional shaking with 19.2 cc of n/100 ammonia solution (30% G), making the total volume 20 cc. Then the mixture was centrifuged and filtered. To 10 cc of the eluate were added 5.0 cc of n/100 acetic acid solution (30% G) and 5.0 cc of 30% glycerine solution making the total volume 20 cc. The eluate of pH 6.2 (=pH of the original enzyme extract) thus obtained was employed in each experiment concerned.

b. The eluate of dried pancreas of pig.

20 cc of original enzyme (30% G) extract IIa were mixed with 2.1 cc of n/100 acetic acid (30% G) solution and 1.9 cc of 30% glycerine solution, and then subjected

to the adsorption with the addition of 16.0 cc of $\text{Al}(\text{OH})_3$ (30% G). As to the adsorption mixture, the pH was ca. 5.0, the glycerine conc. 30%, and the total volume 40 cc; i. e., twice the volume of original enzyme extract. The mixture was well shaken for ca. 5 mins., centrifuged and filtered. The residual solution was discarded.

The adsorbate was subjected to elution for ca. 30 mins. under occasional shaking with 16.8 cc of n/100 ammonia solution (30% G), making the total volume 20 cc. Then the mixture was centrifuged and filtered. To 10 cc of the eluate were added 1.8 cc of n/100 acetic acid (30% G) and 8.2 cc of 30% glycerine solution, making the total volume 20 cc. The eluate of pH 5.9 (=pH of the original enzyme extract) thus obtained was employed in each experiment concerned.

Table I contains a survey of the enzyme materials and enzyme extracts employed, together with the particular data relating to their preparations.

3. Venom solutions.

1% glycerine (30%) solution of the dried venom of *Taiwankobura* [*Naja naja atra* (CANTOR)] was prepared as noted previously,⁴⁾ just before each adsorption experiment.

4. Adsorption agent.

Aluminium hydroxide C_γ was prepared on the 2nd, May 1935, according to the method of WILLSTATTER, KRAUT, and ERBACHER,⁶⁾ and the water suspension contained 184.4 mg Al_2O_3 per 10 cc. Two kinds of the glycerine suspension were employed for the adsorption of the venom after being diluted as follows:

TABLE I.

Survey of the enzyme materials and enzyme extracts employed.

Enzyme material				Enzyme extract				
No of material	Kinds of materials	Date of preparation	Water content %	No of extract	Date of preparation	g of material used	Total volume of mixture cc	Conc. of solvent G %
1	Dried kidney of pig	11/7 1935	10.6	Ia	15/8 1935	2	50	30
	"	"	"	Ib	18/8 1935	2	50	30
	"	"	"	Ic	25/8 1935	2	50	30
2	Dried pancreas of pig	11/7 1935	12.3	IIa	15/8 1935	4	50	30
	"	"	"	IIb	18/8 1935	4	50	30
	"	"	"	IIc	25/8 1935	4	50	30

a. 1 vol. of original water suspension was diluted with 1 vol. of 60 % glycerine solution. Therefore, this suspension contained 92.2 mg Al_2O_3 per 10 cc and 30 % glycerine.

b. 1 vol. of diluted glycerine (30 %) suspension prepared as above was further diluted with the same volume of 30 % glycerine solution. Therefore, this preparation contained 46.1 mg. Al_2O_3 per 10 cc and 30 % glycerine.

B. Experimental Methods.

1. Determination of enzyme activity.

Enzyme activity was determined according to the method described in previous papers.¹⁻⁵⁾

2. Analytical method.

a. *Relation between the amount of venom and the magnitude of its activation power upon the dipeptidase in splitting of AG and LG.*

In the quantitative experiment of the adsorption and the elution of the active constituent of the venom for the activation of dipeptidase, it is necessary, in the first place, to establish this relation. For this purpose, the activation test was made with varying amounts of original venom solution of Taiwankobura upon the LG-splitting by the dipeptidase of pig's kidney as well as upon the AG-splitting by the dipeptidase of pig's pancreas.

According to this, in a certain range such as from $C_v=0$ to $C_v=4.0$ for the former and from $C_v=0$ to $C_v=8.0$ for the latter, the magnitude of the activation power is proportional to the square root of the amount of venom (C_v) employed. Consequently, this relation is expressed by the following equation:—

$$x = k\sqrt{C_v} \dots \dots \dots (1)$$

where x denotes the magnitude of the activation power, expressed in cc of $n/20$ KOH per 2 cc of the digestion mixture; i. e.,

X_{LG} with venom minus X_{LG} without venom, or

X_{AG} with venom minus X_{AG} without venom.

C_v denotes mg of original dried venom per 2cc of the digestion mixture, k denotes the velocity constant of the activation.

For the calculation of the yield (%) of the active constituent of the venom in the residual solution or in the eluate, similar relations were assumed; i.e.,

$$x = k\sqrt{C_v'} \dots\dots\dots(2)$$

$$x = k\sqrt{C_v''} \dots\dots\dots(3)$$

where C_v' and C_v'' are the values corresponding to C_v , the former in the case of using residual venom solution and the latter in the case of using eluate of the venom.

It should be noticed here that these values C_v' and C_v'' are the ones which were calculated from the data which were found in the experiment of each case and the velocity constant which was found in the case $x = k\sqrt{C_v}$. Thus, the calculation was made as follows:—

$$\text{Yield (\%)} \text{ of the active constituent in the residual venom solution} \dots\dots\dots = \frac{C_v'}{C_v} \cdot 100 \text{ (\% for original venom solution)} \dots\dots\dots(4)$$

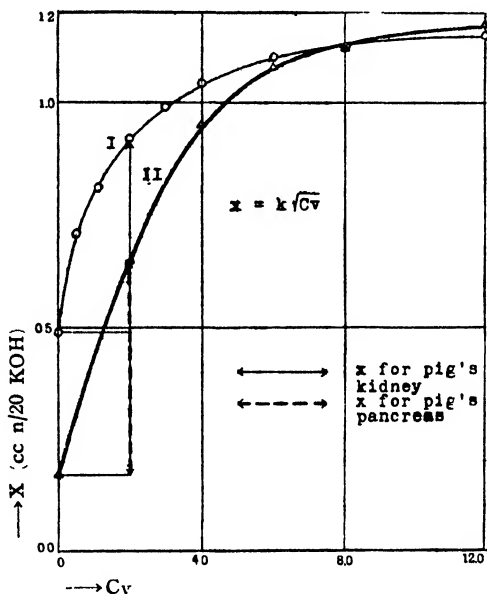
$$\text{Yield (\%)} \text{ of the active constituent in the eluate of the venom} \dots\dots\dots = \frac{C_v''}{C_v} \cdot 100 \text{ (\% for original venom solution)} \dots\dots\dots(5)$$

$$= \frac{C_v''}{C_v - C_v'} \cdot 100 \text{ (\% for adsorbate)} \dots\dots\dots(6)$$

FIG. I.

Curves illustrating the relation between the quantity of venom of *Taiwankobura* and its activation power upon the LG- and AG-cleavages by dipeptidase of the adsorption eluate obtained from the dried kidney or pancreas of pig.

Curves correspond to the figures in table II.



Curves: I, X_{LG} , dried kidney of pig, $C_E=17.74$, $t=60$.

II, X_{AG} , dried pancreas of pig, $C_E=35.08$, $t=120$.

b. Method of adsorption.

The general technique of the adsorption process is to be illustrated by the following typical example which was taken at random from the present experimental data;

10 cc of 1% venom glycerine (30%) solution were mixed with 3.48 cc of n/100 ammonia solution (30% G) and 4.52 cc of 30% glycerine solution and then subjected to the adsorption with the addition of 2.0 cc of $\text{Al}(\text{OH})_3$ C_Y suspension* (30% G). As to the adsorption mixture, the pH was ca. 8.0, glycerine conc. 30%, and the total volume 20 cc; i. e., twice the volume of original venom solution. The mixture was well shaken for 5 mins., centrifuged and filtered. Thus, the residual venom solution was obtained. Then the activation experiment was carried out with the original and residual venom solutions, using 0.5 cc (corresponding to $C_V=2.0$) for the original and 1.0 cc (equivalent to $C_V=2.0$) for the residual respectively.

1 cc of the eluate of pig's kidney (corresponding to $C_F=17.74$) was used as enzyme solution. The results of digestion (60 mins) were obtained as follows:

$$\begin{aligned} X_{LG} &= 0.39 \text{ (without venom)} \\ &= 0.84 \text{ (with original venom)} \\ &= 0.76 \text{ (with residual venom)} \end{aligned}$$

With these data, k was first calculated as follows:

$$\begin{aligned} k &= \frac{x}{1/C_V} \quad \therefore x = k \cdot 1/C_V \text{ (Formula 1, on p. 312)} \\ &= \frac{0.84 - 0.39}{2.0} \\ &= 0.3182 \end{aligned}$$

And then, C_V' was calculated in the following way:

$$\begin{aligned} C_V' &= \frac{x^2}{k^2} \quad \therefore x = k \cdot 1/C_V' \text{ (Formula 2, on p. 313)} \\ &= \frac{(0.76 - 0.39)^2}{(0.3182)^2} \\ &= 1.352 \end{aligned}$$

Therefore, the yield (%) of the constituent active for X_{LG} in the residual solution becomes:

$$\begin{aligned} \% &= \frac{C_V'}{C_V} \cdot 100 \text{ (Formula 4, on p. 313)} \\ &= \frac{1.352}{2.0} \cdot 100 \\ &= 68\% \end{aligned}$$

c. Method of elution.

A typical example of the elution process is noted in the following:

The adsorbate in the above experiment was washed once with 10 cc of 30% glycerine solution which was regulated to pH ca. 8.0 with n/100 ammonia solution (30% G), and then subjected to the elution with the addition of n/100 acetic acid glycerine (30%) solution so as to make the total volume 10 cc (=the volume

* This suspension contained 46.1 mg Al_2O_3 per 10 cc and 30% glycerine.

of original venom solution employed). The mixture was left to stand for ca. 30 mins. under occasional shaking, centrifuged and filtered. The activation experiment was then carried out with 0.5cc of the eluate thus obtained.

The X_{LG} determined was 0.62.

In a similar way to the above example, C_V'' was calculated as 0.522 according to Formula 3 (cf. p. 313), the yield (%), of the constituent active for X_{LG} becomes $0.522/2.0 \cdot 100 = 26\%$ according to Formula 5 (cf. p. 313, and $0.522/2.0 - 1.352 \cdot 100 = 81\%$ according to Formula 6 (cf. p. 313).

C. Symbols.

Symbols except those explained in a previous section of this paper were the same as those employed in previous papers.^{1, 2)}

D. Experimental Results.

The results obtained are given in tables II~VIII and illustrated in figures I~III. Essential points of these results are summarized in the next section.

SUMMARY

1. For the purpose of the quantitative experiment of the adsorption and the elution of the active constituent of the venom for the activation of dipeptidase, was established, in the first place, the relation between the varying amounts of original venom solution of *Taiwankobura* and their activation powers upon the splitting of LG by the dipeptidase of pig's kidney (=case a) or upon the splitting of AG by the dipeptidase of pig's pancreas (=case b). This relation is illustrated in Fig. I.

This relation can also be expressed by the following equation, in a certain range of C_V (cf. symbols on p. 313) such as from 0 to 4.0 in the case (a) and from 0 to 8.0 in the case (b):

$$x = k\sqrt{C_V}$$

k was found to be 0.30 in the case (a) and 0.36 in the case (b).

2. As shown in Fig. II, pH-adsorption-curves of the active constituent of the venom with $Al(OH)_3$ C_T were determined for both cases above named (a, b).

According to this figure, it was observed that, in a certain pH-

range such as from 4.0 to 8.0, the greater the pH-value of the adsorption venom mixture, the more the adsorption of the active constituent of the venom, and at the same time, the adsorption of the active constituent of the venom in the case (b) was far more marked than that in the case (a) throughout all pH-range tested.

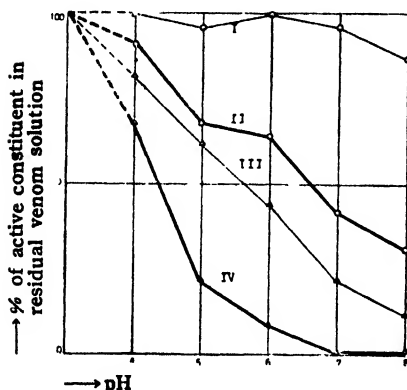
3. Relation between the adsorption of the active constituent of the venom and the amount of adsorption agent $Al(OH)_3$ C_7 was determined and shown by Fig, III. According to this result, the adsorption of the active constituent at pH 8.0 was effective with far less amount of the adsorption agent as compared with that at pH 5.0; the adsorption of the active constituent in the case (b) was effective, than that in the case (a) with far less amount of the adsorption agent.

4. As shown in table VIII, the active constituent of the venom was eluded from the $Al(OH)_3$ -adsorbate with n/100 acetic acid. The yield of the active constituent eluded was 81 % of the amount adsorbed in the case (a) and 40 % of the amount adsorbed in the case (b).

FIG. II.

Curves illustrating the adsorption (%) of the active constituent of the venom with aluminium hydroxide $C\gamma$ at varying pH.

The curves correspond to the figures in table IV.



Curves: The thin curves denote the amount (%) of active constituent of residual venom solution obtained from the adsorption of original venom solution ($C_V=2.0$) with 0.5 cc of $Al(OH)_3 C\gamma$, as observed from the standpoint of its activation power.

I, when test was made upon the splitting of LG by the dipeptidase of pig's kidney.

III, when test was made upon the splitting of AG by the dipeptidase of pig's pancreas.

The thick curves denote the amount (%) of active constituent of residual venom solution obtained from the adsorption of original venom solution ($C_V=2.0$) with 1.5 cc of $Al(OH)_3 C\gamma$, as observed from the standpoint of its activation power.

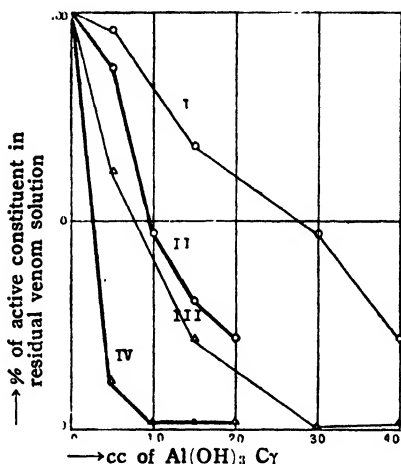
II, when test was made upon the splitting of LG by the dipeptidase of pig's kidney.

IV, when test was made upon the splitting of AG by the dipeptidase of pig's pancreas.

FIG. III.

Curves illustrating the adsorption (%) of the active constituent of the venom of Taiwankobura with varying quantities of aluminium hydroxide $C\gamma$ at pH 5.0 and pH 8.0.

The curves correspond to the figures in table VI.



Curves: The thin curves denote the amount of the active constituent of residual venom solution obtained from the adsorption of original venom solution at pH 5.0, as observed from the standpoint of its activation power.

I, when test was made upon the splitting of LG by the dipeptidase of pig's kidney.

III, when test was made upon the splitting of AG by the dipeptidase of pig's pancreas.

The thick curves denote the amount of the active constituent of residual venom solution obtained from the adsorption of original venom solution at pH 8.0, as observed from the standpoint of its activation power.

II, when test was made upon the splitting of LG by the dipeptidase of pig's kidney.

IV, when test was made upon the splitting of AG by the dipeptidase of pig's pancreas.

TABLE II.

Relation between the quantity of the venom of *Taiwankobura* and its activation power upon the LG- and AG-cleavages by dipeptidase of the adsorption eluate obtained from the dried kidney or pancreas of pig.

Each eluate was prepared from the enzyme extract Ia or IIa in precisely the same way as noted on pp. 310 and 311.

At the determination, $C_E=17.74$, $t=60$ for X_{LG} and $C_E=35.08$, $t=120$ for X_{AG} .

$$x=k\sqrt{C_V} \text{ or } k=\frac{x}{\sqrt{C_V}} \text{ (Formula 1, p. 312).}$$

C _V	Dried kidney of pig				Dried pancreas of pig				
	X _{LG}	x ^{*1)}	√ C _V	k·100 ^{*2)}	X _{AG}	x ^{*1)}	√ C _V	k·100 ^{*2)}	
0	0.49	—	—	—	0.17	—	—	—	
0.5	0.71	0.22	0.7075	31	—	—	—	—	
1.0	0.81	0.32	1.000	32	—	—	—	—	
2.0	0.92	0.43	1.415	30	0.65	0.48	1.415	34	
3.0	0.99	0.50	1.733	29	—	—	—	—	
4.0	1.04	0.55	2.000	28	0.95	0.78	2.000	39	
6.0	1.10	0.61	2.450	(25)	1.08	0.91	2.450	37	
8.0	1.12	0.63	2.830	(22)	1.12	0.95	2.830	34	
12.0	1.15	0.66	3.465	(19)	1.17	1.00	3.465	(29)	
Average.....				30	Average.....				36

*1) $x-X_{LG}$ in case with venom minus X_{LG} in case without venom or X_{AG} in case with venom minus X_{AG} in case without venom. (cf. p. 312).

*2) Values of k 100 in parentheses have not been included in calculating average values.

TABLE III.

Adsorption of the active constituent of the venom of *Taiwankobura* with aluminium hydroxide C_V at varying pH.

As enzyme solution, each eluate was prepared from the enzyme extract Ib or IIb in precisely the same way as noted on pp. 310 and 311.

At the determination, $C_E=17.74$, $t=60$ for X_{LG} and $C_E=35.08$, $t=240$ for X_{AG} .

pH	Composition of venom adsorption mixture per 5 cc						X _{LG}			X _{AG}		
	1% venom sol. (30% G) cc	Acetic acid solution (30% G) cc		n/100 ammonia sol. (30% G) cc	30% glycerine sol. cc	Al(OH) ₃ C _γ suspension* ¹⁾ (30% G) cc	Dried kidney of pig (eludate)			Dried pancreas of pig (eludate)		
		n/10	n/100				C _V =0	0.5 cc orig. venom sol. (C _V =2.0)	1.0 cc resid. venom sol.* ²⁾	C _V =0	0.5 cc orig. venom sol. (C _V =2.0)	1.0 cc resid. venom sol.* ²⁾
4.0	2.5	0.81	0	0	1.19	0.5	0.44	0.89	0.89	0.33	1.49	1.38
5.0		0	0.50	0	1.50				0.88			1.24
6.0		0	0.10	0	1.90				0.88			1.10
7.0		0	0	0.35	1.65				0.89			0.88
8.0		0	0	0.87	1.13				0.86			0.73
4.0	2.5	0.81	0	0	0.19	1.5	0.44	0.89	0.87	0.33	1.49	1.29
5.0		0	0.50	0	0.50				0.81			0.87
6.0		0	0.10	0	0.90				0.80			0.68
7.0		0	0	0.35	0.65				0.73			0.48
8.0		0	0	0.87	0.13				0.69			0.48

*1) Al(OH)₃ C_γ suspension contained 46.1 mg. Al₂O₃ per 10 cc.

*2) 1.0 cc of residual venom solution corresponds to 0.5 cc of original venom solution.

TABLE IV.

Calculation of adsorption of the active constituent of the venom of Taiwankobura with aluminum hydroxide C_γ at varying pH.

According to Formula 2 on p. 313 and Formula 4 on p. 313,

$$\% = \frac{C_V'}{C_V} \cdot 100 = \frac{C_V'}{2.0} \cdot 100 \text{ where } C_V' = \frac{x'}{k^2} \quad \therefore x = k^2 \cdot C_V'; \quad x = X_{LG} \text{ in case with venom minus } X_{LG} \text{ in case without venom or } X_{AG} \text{ in case with venom minus } X_{AG} \text{ in case without venom; } k = \frac{x}{C_V}.$$

Calculation was made from the figures in table III.

pH	Al(OH) ₃ C _γ * ¹⁾ cc	Activation power of residual venom solution							
		Dried kidney of pig (eludate)				Dried pancreas of pig (eludate)			
		x	k* ²⁾	C _V '	%	x	k* ³⁾	C _V '	%
4.0	0.5	0.45	0.3182	2.000	100	1.05	0.8202	1.638	82
5.0		0.44		1.912	96	0.91		1.231	62
6.0		0.45		2.000	100	0.77		0.881	44
7.0		0.44		1.912	96	0.55		0.449	22
8.0		0.42		1.742	87	0.40		0.238	12
4.0	1.5	0.43	0.3182	1.826	91	0.96	0.8202	1.369	68
5.0		0.37		1.352	68	0.54		0.433	22
6.0		0.36		1.280	64	0.35		0.182	9
7.0		0.29		0.830	42	0.15		0.033	2
8.0		0.25		0.617	31	0.15		0.033	2

*1) $\text{Al}(\text{OH})_3$ C_γ suspension contained 46.1 mg Al_2O_3 per 10 cc.

$$*2) k = \frac{x}{\sqrt{C_V}} = \frac{0.89 - 0.44}{\sqrt{2.0}} = 0.3182.$$

$$*3) k = \frac{x}{\sqrt{C_V}} = \frac{1.49 - 0.33}{\sqrt{2.0}} = 0.8202.$$

TABLE V.

Adsorption of the active constituent of the venom of *Taiwankobura* with varying quantities of aluminium hydroxide C_γ at pH 5.0 and 8.0.

As enzyme solution, each eluate was prepared from the enzyme extract Ib or IIb in precisely the same way as noted on pp. 310 and 311.

At the determination, $\text{C}_E = 17.74$, $t = 60$ for X_{LG} and $\text{C}_E = 35.08$, $t = 240$ for X_{AG} .

Composition of venom adsorption mixture per 5 cc						X_{LG}			X_{AG}		
pH	1% venom sol. (30% G) cc	n/10 acetic acid sol. (30% G) cc	n/100 ammonia sol. (30% G) cc	30% glycerine sol. cc	$\text{Al}(\text{OH})_3$ suspension (30% G) cc	Dried kidney of pig (eluate)			Dried pancreas of pig (eluate)		
						$\text{C}_V = 0$	0.5 cc orig. venom ($\text{C}_V = 2.0$)	1.0 cc resid. venom	$\text{C}_V = 0$	0.5 cc orig. venom ($\text{C}_V = 2.0$)	1.0 cc resid. venom
5.0	2.5	0.50	0	1.5	0.5*	0.44	0.89	0.88	0.33	1.49	1.24
				0.5	1.5*			0.81			0.87
				0.5	1.5**			0.75			0.47
				0	2.0**			0.65			0.48
8.0	0	0.87	0.87	1.13	0.5*	0.44	0.89	0.86	0.33	1.49	0.73
				0.63	1.0			0.75			0.50
				0.13	1.5			0.69			0.48
				0.63	1.0**			0.65			0.48

* $\text{Al}(\text{OH})_3$ C_γ suspension contained 46.1 mg Al_2O_3 per 10 cc.

** $\text{Al}(\text{OH})_3$ C_γ suspension contained 92.2 mg Al_2O_3 per 10 cc.

TABLE VI.

Calculation of adsorption of the active constituent of the venom of *Taiwankobura* with varying quantities of aluminium hydroxide C_γ at pH 5.0 and 8.0.

According to Formula 2 on p. 313 and Formula 4 on p. 313,

$$\% = \frac{C_V'}{C_V} \cdot 100 = \frac{C_V'}{2.0} \cdot 100 \text{ where } C_V' = \frac{x^2}{k^2} \quad \therefore x = k\sqrt{C_V'}; x = \text{X}_{\text{LG}} \text{ in case with venom minus X}_{\text{LG}} \text{ in case without venom or X}_{\text{AG}} \text{ in case with venom minus X}_{\text{AG}} \text{ in case without venom; } k = \frac{x}{\sqrt{C_V}}.$$

Calculation was made from the figures in table V.

pH	Al(OH) ₃ C _V ^{*1)} cc	Activation power of residual venom solution							
		Dried kidney of pig (eludate)				Dried pancreas of pig (eludate)			
		x	k ^{*2)}	C _V '	%	x	k ^{*3)}	C _V '	%
5.0	0.5	0.44	0.3182	1.912	96	0.91	0.8202	1.231	62
	1.5	0.37		1.352	68	0.54		0.433	22
	3.0	0.34		1.141	57	0.14		0.029	1
	4.0	0.21		0.435	22	0.15		0.033	2
8.0	0.5	0.42	0.3182	1.742	87	0.40	0.8202	0.238	12
	1.0	0.31		0.949	47	0.17		0.042	2
	1.5	0.25		0.617	31	0.15		0.033	2
	2.0	0.21		0.435	22	0.15		0.033	2

*1) 1 cc of Al(OH)₃ C_V suspension corresponds to 4.61 mg Al₂O₃.

$$*2) k = \frac{x}{\sqrt{C_V}} = \frac{0.89-0.44}{\sqrt{2.0}} = 0.3182.$$

$$*3) k = \frac{x}{\sqrt{C_V}} = \frac{1.49-0.33}{\sqrt{2.0}} = 0.8202,$$

TABLE VII.

Elution of the active constituent of the venom of Taiwankobura from the aluminium hydroxide C_V-adsorbate with dilute acetic acid.

As enzyme solution, each eludate was prepared from the enzyme extract Ic or IIc in precisely the same way as noted on pp. 310 and 311.

At the determination, C_E=17.74, t=60 for X_{LG} and C_E=35.08, t=240 for X_{AG}.

As venom solution, were employed original, residual, and eludate which were prepared in the following way:

10 cc of 1% of original venom solution+3.48 cc of n/100 ammonia solution (30% G)+2.0 cc of Al(OH)₃ C_V suspension (the suspension contained 46.1 mg Al₂O₃ per 10 cc, glyc. conc. of which was 30%)+4.52 cc of 30% glyc.→20 cc (pH 8.0), centrifuged and filtered. Thus the residual venom solution was obtained. The adsorbate was washed once with ca. 10 cc of 30% glyc. solution which was regulated to pH 8.0 with n/100 ammonia solution (30% G), to which n/100 acetic acid solution (30% G) was added so as to make the total volume 10 cc. After ca. 30 mins. standing under occasional shaking, centrifuged and filtered, thus the eludate was obtained.

At the determination, three components of the venom (original solution, residual solution, and eludate) were immediately used without standing.

Venom solution		X _{LG}	X _{AG}
Kinds of venom solutions	cc used per 5 cc of digestion mixture	Dried kidney of pig (eluate)	Dried pancreas of pig (eluate)
Original	0 (C _V =0)	0.39	0.22
	0.5 (C _V =2.0)	0.94	1.30
Residual	1.0*	0.76	0.63
Eluate	0.5**	0.62	0.85
	1.0	0.69	1.17
	1.5	0.79	1.39

* 1 cc of residual venom solution corresponds to 0.5 cc of original venom solution.

** 0.5 cc of eluate corresponds to 0.5 cc of original venom solution.

TABLE VIII.

Calculation of the active constituent of the venom of *Taiwankobura* from the aluminium hydroxide C_V-adsorbate with dilute acetic acid.

In the case of residual venom solution, % was calculated according to Formula 2 on p. 313 and Formula 4 on p. 313:

$$\% = \frac{C_V'}{C_V} \cdot 100 - \frac{C_V'}{2.0} \cdot 100 \quad \text{where } C_V' = \frac{x^2}{k^2} \quad \therefore x = k \sqrt{C_V'}; \quad x = X_{LG} \text{ in case with venom minus } X_{LG} \text{ in case without venom or } X_{AG} \text{ in case with venom minus } X_{AG} \text{ in case without venom; } k = \frac{x}{\sqrt{C_V}}.$$

Similarly, in the case of eluate, % was calculated according to Formulæ 3, 5, and 6 on p. 313,

$$\% = \frac{C_V''}{C_V} \cdot 100 = \frac{C_V''}{2.0} \cdot 100 \dots (a) \quad (\% \text{ for original venom solution employed}).$$

or

$$\% = \frac{C_V''}{C_V - C_V'} \cdot 100 = \frac{C_V''}{2.0 - C_V'} \cdot 100 \dots (b) \quad (\% \text{ for adsorbate}).$$

Calculation was made from the figures in table VII.

Venom solution		Dried kidney of pig (eluate)				Dried pancreas of pig (eluate)			
Kinds of venom solutions	cc used per 5 cc of digestion mixture	x	k*1)	C _V	%	x	k*2)	C _V	%
Original	0.5	0.45	0.3182	2.000 (C _V)	100	1.08	0.7636	2.000 (C _V)	100
Residual	1.0	0.37		1.352 (C _V ')	68	0.41		0.288 (C _V ')	14
Eluate	0.5	0.23		0.522 (C _V '')	26*3) 81*4)	0.63		0.680 (C _V '')	34*3) 40*4)
	1.0	0.30		0.878 (C _V ''×2)	22*3) 68*4)	0.95		1.547 (C _V ''×2)	39*3) 45*4)
	1.5	0.40		1.560 (C _V ''×3)	26*3) 80*4)	1.17		2.347 (C _V ''×3)	39*3) 46*4)

$$*1) k = \frac{x}{C_V} = \frac{0.84 - 0.39}{2.0} = 0.3182. \quad *2) k = \frac{x}{C_V} = \frac{1.30 - 0.22}{\sqrt{2.0}} = 0.7636.$$

*3) % was calculated from (a) above stated.

*4) % was calculated from (b) above stated.

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PART VII.

On the Variation of the Activation Power of the Venoms of Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)] and Taiwankobura [*Naja naja atra* (CANTOR)] upon the Enzymatic Cleavage of Leucylglycine according to the Successive Squeezing of the Venom.

(With 2 Text-Figures)

Yoshio TSUCHIYA

(Accepted for publication, April 4, 1936)

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INTRODUCTION

In the present investigation, test was made on the question how the activation power of the venom upon the enzymatic cleavage of leucylglycine varies according to the venom samples which were

taken by squeezing the venom glands of the same groups of snakes successively on different dates at intervals of two weeks.

For this purpose, test was made with three groups of snakes as noted on p. 327 (cf. table I on p. 328), using as enzyme material an eludate which was obtained from the kidney of pig.

The results are summarized on p. 329.

The author wishes to express his sincere thanks to Prof. Dr. Masakazu SATO., for his kind advice and encouragement throughout this investigation. He is also indebted to Mr. H. KAMACHI, for the assistance he has given to the present work.

EXPERIMENTAL PART

A. Preparations Employed.

1. *LG-buffer solution.*

LG and LG-buffer solution were prepared in exactly the same way as described in a previous paper.¹⁾

2. *Enzyme material.*

As enzyme material, was employed dried powder of pig's kidney, which was prepared 16/1 1936, according to the acetone-ether treatment of WILLSTÄTTER and WALDSCHMIDT-LEITZ.²⁾

The water content of this powder amounted to 6.4 %.

3. *Enzyme solution (eludate)*

a. Original enzyme extract.

Original enzyme extract was prepared 20/1 1936, as noted below.

2 g. of the dried powder of pig's kidney were well extracted with 30 % glycerine solution, making the total volume 50 cc. The mixture was centrifuged and filtered. Clear original extract was thus obtained.

b. Enzyme solution (eludate)

20 cc of original enzyme extract were mixed with 7.0 cc of n/10 acetic acid solution (30 % G) and 5.0 cc of 30 % glycerine solution, and the mixture was subjected to the adsorption with the

addition of 8.0 cc of $\text{Al}(\text{OH})_3$ $\text{C}\gamma$ suspension* (30 % G). As to the mixture, the pH was thus 5.0, glycerine conc. 30 %, and the total volume twice that of original extract. Then the mixture was well shaken and left to stand for ca. 5 mins., centrifuged and filtered. The residual solution was discarded. Then the residue was subjected to the elution for ca. 30 mins. under occasional shaking with the addition of 18.5 cc of n/100 ammonia solution (30 % G), making the total volume 20 cc. The mixture was centrifuged and filtered.

To 10 cc of the eludate were added 2.08 cc of 30 % glycerine solution, making the total volume 20 cc. The eludate of pH 7.0 thus obtained was used in each experiment without standing.

4. *Venom-samples.*

The venom of Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)] and Taiwankobura [*Naja naja atra* (CANTOR)] were employed.

Three snakes of Taiwanhabu were kept without food (=Group A).

As to the Taiwankobura, three snakes were kept without food (=Group B) and two other snakes were kept with food (=Group C).

A series of venom samples was taken, by squeezing the venom-glands of the snakes of each group on different dates at intervals of two weeks.

In table I is given a survey of the venom-samples taken and the particular data relating to their preparations.

B. Experimental Methods.

The determination of enzyme activity was carried out in the same way as that previously noted.¹⁾

Throughout all digestion experiments, the conditions were kept as follows :

This suspension was prepared 21/12 1935, 1 cc of which contained 3.15 mg. Al_2O_3 .

Substrate conc.=0.1 mol.,
 Glycerine conc.=15 %,
 pH=8.0±0.05 (ammonia ammonium chloride buffer),
 Digestion for 1 hr. at 40°.

In the case of the digestion with venom, each 5.0 mg. of the venom of Taiwanhabu or 2.5 mg. of the venom of Taiwankobura were weighed and dissolved in 1.5 cc or 0.5 cc of 30 % glycerine solution respectively, and then added to the digestion liquid (=5.0 cc).

With regard to the detailed process of digestion with and without the venom, reference should be made to the general process (p. 141) in a previous paper.¹⁾

C. Symbols.

The symbols used in the present investigation were the same as those previously noted.¹⁾

TABLE I.

Survey of the venoms of Taiwanhabu and Taiwankobura sampled.

Kinds of snakes	Groups	No of venom samples	Condition of feeding	Number of snakes in each group	Venom of snake		
					Date of taking the venom by squeezing the venom glands	Fresh g	Dried g
Taiwanhabu	A	1a	Without food	3	15/10 1935	1.8478	0.6340
		2a			29/10	0.9882	0.2775
		3a			12/11	1.0499	0.2975
		4a			26/11	1.2977	0.3690
		5a			10/12	0.9805	0.2759
		6a			21/12	0.5004	0.1300
Taiwankobura	B	1b	Without food	3	15/10 1935	0.7311	0.1656
		2b			29/10	0.7815	0.2427
		3b			*12/11	0.3449	0.0938
		4b			26/11	0.4350	0.1159
		5b			10/12	0.2228	0.0552
		6b			24/12	0.2690	0.0516
	C	1c	With food	2	15/10 1936	0.5402	0.1296
		2c			29/10	0.4056	0.1182
		3c			12/11	0.2023	0.0207
		4c			26/11	0.3179	0.0611
		5c			10/12	0.2433	0.0618

* One snake of the three died at this period.

D. Experimental Results.

Results obtained are clearly shown in tables II and III, and also illustrated in figures I and II.

SUMMARY

1. In the case of group A, i. e., the snakes of Taiwanhabu which were kept without food;—the activation power of the venom was found to be approximately constant within the first 8 weeks but thereafter it was gradually decreasing.

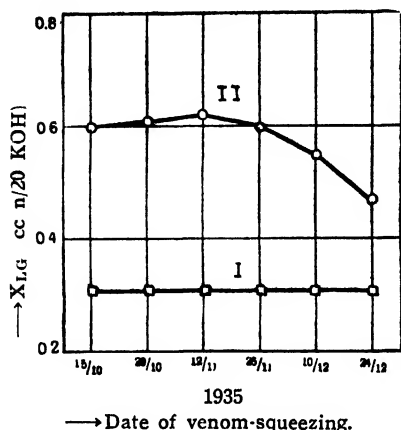
2. In the case of group B, i. e., the snakes of Taiwankobura which were kept without food;—the activation power of the venom was constant within the first 4 weeks; it then became somewhat higher for 4 weeks, and thereafter it gradually decreased.

3. In the case of group C, i. e., the snakes of Taiwankobura which were kept with food;—the activation power of the venom was constant within the first 2 weeks; it then became somewhat higher for a time, and later it gradually decreased.

FIG. I

Curves illustrating the variation of the activation power of the venom of Taiwanhabu according to different venom samples which were taken by squeezing the venom-glands of the same group of snakes on different dates at intervals of two weeks.

The curves correspond to the figures in table II.

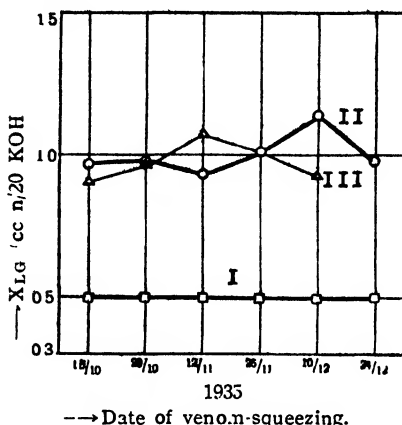


Curves: I, $C_E=3.74$, $C_V=0$.
II, Without food, $C_E=3.74$, $C_V=2.0$.

FIG. II.

Curves illustrating the variation of the activation power of the venom of Taiwankobura according to different venom-samples which were taken by squeezing the venom-glands of the same groups of snakes on different dates at intervals of two weeks.

The curves correspond to the figures in table III.



Curves: I, $C_E=3.74$, $C_V=0$.
II, Without food, $C_E=3.74$, $C_V=1.0$.
III, With food, $C_E=3.74$, $C_V=1.0$.

No of venom samples	X_{LG}			
	$C_V=0$		$C_V=2.0$	
1a	0.30	0.31	0.60	0.60
	0.32		0.60	
2a		"	0.59	0.61
			0.62	
3a		"	0.60	0.62
			0.63	
4a		"	0.59	0.60
			0.61	
5a		"	0.54	0.55
			0.56	
6a		"	0.45	0.47
			0.48	

TABLE II.

Variation of the activation power of the venom of Taiwanhabu according to different venom samples which were taken by squeezing the venom-glands of the same group of snakes on different dates at intervals of two weeks.

The activation power was tested upon the LG-splitting by dipeptidase of the eludate of dried kidney of pig. The snakes of this group A were kept without food. At the determination, $C_E=3.74$,

TABLE III.

Variation of the activation power of the venom of *Taiwankobura* according to different venom samples which were taken by squeezing the venom glands of the same groups of snakes on different dates at intervals of two weeks.

The activation power was tested upon the LG-splitting by dipeptidase of the eludate of dried kidney of pig.

The snakes of group B were kept without food and those of group C were kept with food. At the determination $C_E=3.74$.

Condition of feeding	Group	No of venom samples	X _{LG}			
			C _V =0		C _V =1.0	
Without food	B	1b	0.50	0.50	0.98	0.97
			0.50		0.96	
		2b		"	0.99	0.98
					0.97	
		3b		"	0.93	0.93
					0.93	
		4b		"	1.02	1.01
					0.99	
		5b		"	1.15	1.14
					1.12	
		6b		"	1.00	0.98
					0.96	
With food	C	1c	0.50	0.50	0.90	0.91
			0.50		0.91	
		2c		"	0.96	0.97
					0.98	
		3c		"	1.05	1.07
					1.08	
		4c		"	1.03	1.02
					1.00	
		5c		"	0.92	0.93
					0.93	

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Ueber Kobaltiammine, die quartäre Ammoniumbasen koordinieren.*

(Mit 19 Figuren im Text.)

Yuichi NAKATSUKA und Yôka ISHIGAKI.

(Accepted for publication, Sept. 28, 1933.)

Während seines Studium über Aquokobaltiammine hat einer von uns (NAKATSUKA) zufällig gefunden, dass das Absorptionsspektrum des Aquopentamminkobaltichlorids in einer ammoniakalischen Lösung ganz verschieden ist von der wässrigen Lösung des Hydroxopentaminsalzes mit demselben Kobaltgehalt.

In der vorliegenden Arbeit haben wir einige Untersuchungen über aminhaltige Lösungen von Aquokobaltiamminen im allgemeinen durchgeführt, und dadurch sind wir auf den Schluss angekommen, dass in solchen Lösungen sich diejenigen Salze noch unbekannter Reihe bilden, bei denen statt koordinativ gebundener Wassermoleküle quartäre Ammoniumhydroxyde sich am Kobaltatom koordinieren.

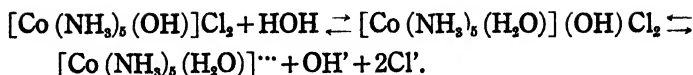
Absorptionskurven wässriger bzw. aminhaltiger Lösungen von Aquokobaltiamminen und verwandter Salze.

$[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ reagiert in wässriger Lösung alkalisch; das beruht, wie allgemein angenommen, auf folgendem¹⁾:

* Ein Teil dieser Arbeit wurde schon veröffentlicht, siehe Y. NAKATSUKA, Journ. Chem. Soc. Japan, 48, 66 (1927) (Japanisch).

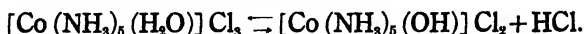
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[Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ., Formosa, Japan, Vol. X, No. 1, November 1933.]

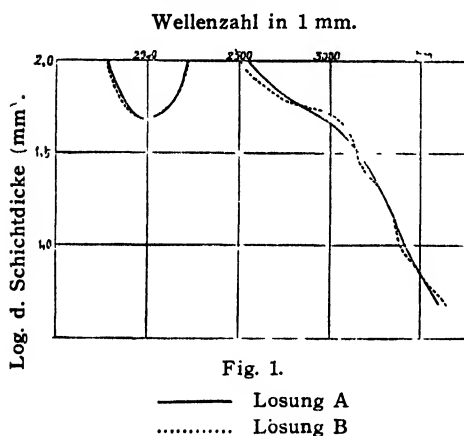


Wenn man deswegen eine Lösung von $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ mit einer Säure abstumpft, bildet sich das $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz.

Ebenfalls ist die saure Reaktion der Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ durch das folgende Gleichgewicht bedingt:



Daraus ist folgendes gleicherweise leicht vorstellbar: Werden zu einer Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ OH-Ionen zugesetzt, so wird sich das Hydroxosalz bilden.



tionsspektrum. Fig. 1 zeigt Absorptionskurven folgender Lösungen:

Lösung A $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2^{(2)}$ 0,01 norm.³⁾

wässrige Lösung

Lösung B $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ 0,01 norm. in 0,1 norm.

Na_2CO_3 -Lösung⁴⁾.

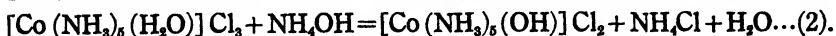
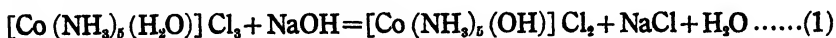
Wenn aber statt NaOH bzw. Na_2CO_3 NH_3 -Lösung verwendet wird, findet man eine Absorptionskurve ganz verschiedener Art im

2) Dieses Salz hat gewöhnlich ein Mol. Kristallwasser. In der vorliegenden Arbeit wird es aber einfachheitshalber stets ohne Kristallwasser angegeben.

3) Normalität der Kobaltiammine bezieht sich stets auf Kobaltatom, also z.B. 0,01 norm. $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ -Lösung ist 0,005 molar.

4) Die NaOH-Lösung des Kobaltiammins ist nicht sehr beständig, und deswegen wurde Na_2CO_3 fast immer statt NaOH verwendet.

ultravioletten Gebiet (siehe Fig. 2). Nun wurde bisher doch allgemein angenommen, dass solche Lösungen stets $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{-Salz}$ geben, wie folgende Gleichungen zeigen:



Die Ursache des eben angegebenen Unterschieds der Absorption kann man verschieden deuten, jedoch kann die zutreffende Erklärung nur folgende sein, was weiter unten näher begründet wird⁵⁾: Wenn man zu einer Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ NH_4OH zusetzt, so bildet sich nicht

$[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$
(wenigstens hauptreaktionsweise nicht), sondern eine noch unbekannte Art Ammin. Also die oben angegebene Gleichung (2) entspricht nicht dem tatsächlichen Reaktionsverlauf.

In Fig. 2 sind Absorptionskurven folgender Lösungen dargestellt:

Lösung C $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ 0,01 norm. in 0,01 norm. NH_3 -Lösung

Lösung D $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ 0,01 norm. in 0,1 norm. NH_3 -Lösung

Lösung E $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ 0,01 norm. in 1 norm. NH_3 -Lösung.

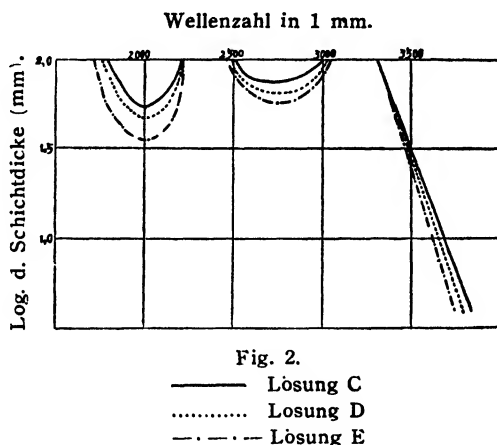


Fig. 2.

— Lösung C
..... Lösung D
- · - · - Lösung E

Die Absorptionskurve desselben Chlörids in der wässrigen Lösung wird in Fig. 4 wiedergegeben. Daraus lässt sich deutlich erkennen, dass die Absorptionsbanden um so tiefer werden, je konzentrierter NH_4OH in der Lösung ist. Dieselbe Vertiefung wird festgestellt

5) Alle anderen denkbaren Ursachen, die keineswegs richtige Erklärungen geben, werden weiter unten beschrieben, siehe S. 9f.

wenn man die Lösung während der Aufnahme des Spektrums mit Eis kühlt (Die Absorptionskurve der erkalteten Lösung ist hier nicht gezeigt). Durch das oben beschriebene wird man sich leicht überzeugen, dass in einer ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ folgendes Gleichgewicht stattfindet:



Das Gleichgewicht verschiebt sich nach rechts mit der Zunahme der NH_4OH -Konzentration und auch mit der Abnahme der Temperatur.

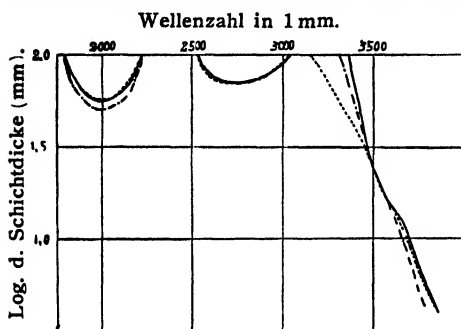


Fig. 3.

— Lösung F
 Lösung G
 - · - · - Lösung H

Andere Aquopentamminsalze, wie das Sulfat, Nitrat und Oxalat reagieren auch mit NH_4OH ganz genau wie das Chlorid. In Fig. 3 sind Absorptionskurven folgender Lösungen angegeben (vgl. Fig. 2 und 4):

Lösung F $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ 0,01 norm. in 0,1 norm.

NH_3 -Lösung

Lösung G $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})](\text{NO}_3)_3$ 0,01 norm. in 0,1 norm.

NH_3 -Lösung

Lösung H $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{C}_2\text{O}_4)_3$ 0,01 norm. in 0,1 norm.

NH_3 -Lösung.

Nur die Endabsorption des Nitrats zeigt eine geringe Abweichung, aber als Ganzes können dennoch alle diese Kurven mit denen des Chlorids als identisch angesehen werden. Die Bildung des betreffenden Ammins beruht also lediglich auf der Reaktion von NH_4OH mit dem $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Radikal. Säurereste der Salze spielen dabei keine Rolle.

Zum Vergleich seien hier noch Absorptionskurven folgender Lösungen in Fig. 4 angegeben:

Lösung I $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ 0,01 norm. wässrige Lösung

Lösung K $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ 0,01 norm. wässrige Lösung

Lösung L $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})] (\text{NO}_3)_3$ 0,01 norm. wässrige Lösung.

Es ist doch nun sehr wahrscheinlich, dass das koordinativ gebundene Wassermolekül vom $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Radikal bei der Bildung des betreffenden Ammins eine grosse Rolle spielt. Um diese Ansicht zu klären, wurden folgende Ammine, die kein koordinatives Wasser in den Komplex-

radikalen enthalten, daraufhin untersucht, ob sie auch mit NH_4OH ähnliche Reaktion geben können oder nicht:

- | | |
|---|---|
| 1) $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ | 2) $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ |
| 3) $[\text{Co}(\text{NH}_3)_5](\text{NO}_3)_3$ | 4) $[\text{Co}(\text{NH}_3)_5(\text{NCS})]\text{SO}_4$ |
| 5) $[\text{Co}(\text{NH}_3)_5(\text{NO}_2)]\text{Cl}_2$ | 6) $[\text{Co}(\text{NH}_3)_4(\text{CO}_3)]_2\text{SO}_4$ |
| 7) $[\text{Co}(\text{NH}_3)_2(\text{NO}_2)_4]\text{NH}_4$ | |

Es ergab sich, dass alle diese Ammine, ausser $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$, nicht mit NH_4OH reagieren. Die ammoniakalische Lösung von $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ zeigte dagegen dieselbe Absorptionskurve wie die ammoniakalische $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ -Lösung. Diese Tatsache braucht jedoch deshalb nicht besonders berücksichtigt zu werden, da $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ in alkalischer Lösung glatt in $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ übergeht und infolgedessen dessen Absorptionskurve ergeben muss. Wenn also ein Ammin kein Wassermolekül koordinativ gebunden enthält, oder wenn ein anderes koordinatives Atom bzw. eine Atomgruppe von einem Ammin in der alkalischen Lösung fest gebunden ist und durch Wasser nicht substituiert wird, dann entsteht die betreffende neue Art des Ammins nicht.

Wenn man einerseits zu der betreffenden Ammin-Lösung d. h. der ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ Alkohol fügt,

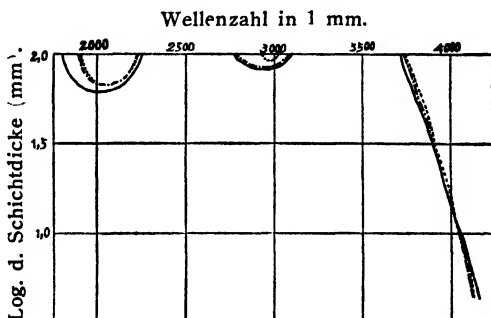


Fig. 4.

— Lösung I
 Lösung K
 - · - · - Lösung L

so zersetzt sich dieses Ammin und es fällt $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ aus⁶⁾; dies zeigt, dass sich $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ nur bei der Fällung bildet. Wird die Lösung andererseits in der Kälte mit HCl neutralisiert, so entsteht, wie allgemein bekannt, $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_2$ wieder. Durch diese beiden Reaktionen lässt sich erklären, dass das zentrale Kobaltatom des betreffenden Ammins wie das Mutteraquosalz, fünf Moleküle NH_3 koordiniert, dass aber an der sechsten Koordinationsstelle eine mit einem Sauerstoffatom gebundene Atomgruppe steht nach folgendem Schema:

$(\text{NH}_3)_5\text{Co}(\text{OX})$, wo X noch unbekannt ist.

Im folgenden werden nun die Reaktionen von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz mit Aminen bzw. Aminoverbindungen anstelle von Ammoniak beschrieben. Folgende Verbindungen wurden dazu verwendet:

- | | |
|--|---|
| 1) Aethylendiamin $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ | 2) Methylamin CH_3NH_2 |
| 3) Diäthylamin $(\text{C}_2\text{H}_5)_2\text{NH}$ | 4) Trimethylamin $(\text{CH}_3)_3\text{N}$ |
| 5) Tetramethylammoniumhydroxyd $(\text{CH}_3)_4\text{NOH}$ | |
| 6) Piperidin $\text{C}_5\text{H}_{11}\text{N}$ | 7) Pyridin $\text{C}_5\text{H}_5\text{N}$ |
| 8) Harnstoff $\text{CO}(\text{NH}_2)_2$ | 9) Urethan $\text{NH}_2\text{COOC}_2\text{H}_5$ |

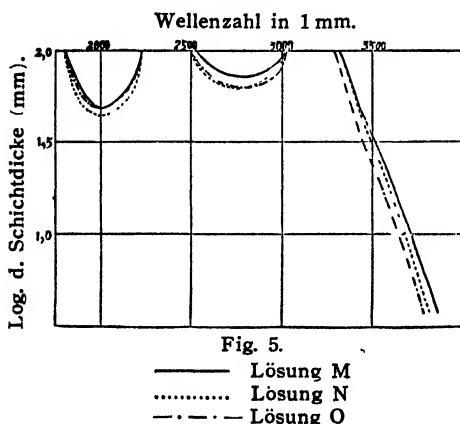


Fig. 5.

— Lösung M
 Lösung N
 - · - · - Lösung O

Absorptionskurven folgender Lösungen wiedergegeben (vgl. Fig. 2 und 3):

6) A. WERNER, Ber. 40, 4106 (1907).

Die beiden letzten, Harnstoff und Urethan, geben keine Reaktion mit $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz; alle anderen Amine reagieren jedoch, und zwar geben die Amine von 1) bis 6) genau dieselben Absorptionskurven wie in dem Falle, wo Ammoniak verwendet wurde. In Fig. 5 sind Ab-

Lösung M	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$	0,01 norm. in 0,01 norm. $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$ -Lösung
Lösung N	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$	0,01 norm. in 0,01 norm. $(\text{C}_2\text{H}_5)_2\text{NH}$ -Lösung
Lösung O	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$	0,01 norm. in 0,01 norm. $(\text{CH}_3)_4\text{NOH}$ -Lösung.

Bei Pyridin greift die Absorption desselben auf die des Ammins über und deswegen kann man die Endabsorption nicht bemerken, aber man findet noch die zwei charakteristischen Banden des betreffenden Ammins. Diese Banden sind jedoch bei Pyridin nicht so tief wie bei den anderen. Dies ist durch die Unvollständigkeit der Reaktion bedingt, welche darauf zu beruhen scheint, dass Pyridin als Base nicht stark genug reagiert; seine Ionisationskonstante liegt in der Grössenordnung 10^{-9} , während alle anderen reagierbaren Amine viel stärker sind und Dissoziationskonstanten von der Ordnung 10^{-5} haben. Über diese Beziehung wird nachher nochmals die Rede sein (siehe S. 20).

Wir haben aus dem oben beobachteten den Schluss bezogen, dass das koordinative Wassermolekül eine grosse Rolle spielt, wenn $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz mit Aminen reagiert. Ganz gleicherweise finden solche Reaktionen auch bei $[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]$ - und $[\text{Co}(\text{NH}_3)_3(\text{H}_2\text{O})_3]$ -Salzen statt. Diese beiden Arten Ammine geben in der Aminlösung überhaupt sehr ähnliche Absorptionen mit $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz. Absorptionsspektren folgender Lösungen sind aufgenommen worden:

Lösung P	$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]\text{Cl}_3$	0,01 norm. in 0,1 norm. NH_3 -Lösung
Lösung Q	$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]_2(\text{SO}_4)_3$	0,01 norm. in 0,1 norm. NH_3 -Lösung
Lösung R	$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]_2(\text{SO}_4)_3$	0,01 norm. in 0,1 norm. $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$ -Lösung
Lösung S	$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]\text{Cl}_3$	0,01 norm. in 1,0 norm. NH_3 -Lösung
Lösung T	$[\text{Co}(\text{NH}_3)_3(\text{H}_2\text{O})_3]\text{Cl}_3$	0,01 norm. in 1,0 norm. NH_3 -Lösung.

Die Absorptionskurven der Lösungen P und R sind in Fig. 6 und die von S und T in Fig. 7 angegeben (vgl. Fig. 2, 3 und 5). Alle diese Kurven sind mit denen der $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{-Salze}$ in der Aminlösung ganz analog, aber im Vergleich zu diesen hat eine Verschiebung der beiden Absorptionsmaxima nach Rot und eine Erhöhung der ultravioletten

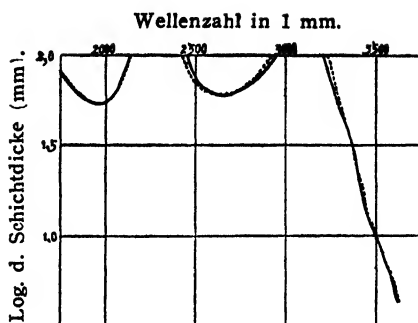


Fig. 6.

— Lösung P
 Lösung R

Absorption stattgefunden, was in um so grösserem Masse der Fall ist, je mehr H_2O -Moleküle in den Mutterkomplexradikalen vorhanden sind.

Die ammoniakalische Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_2$ ist beständig und lange Zeit haltbar, dagegen ist eine gleiche Lösung von $[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]\text{Cl}_2$ viel unbeständiger und trübt sich nach kurzer

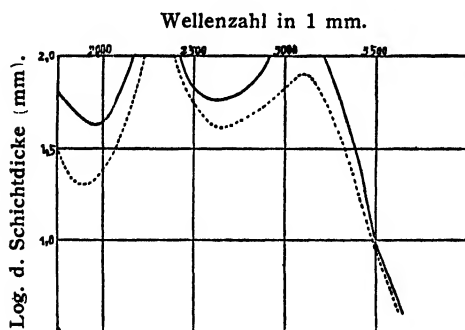


Fig. 7.

— Lösung S
 Lösung T

Zeit, wenn man keinen Ueberschuss an Ammoniak nimmt. Das System $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_2\text{—NH}_4\text{OH}$ ist noch unbeständiger, und man muss daher einen sehr grossen Ueberschuss an Ammoniak anwenden, damit keine Trübung entsteht. Deswegen ist es fast unmöglich eine Aufnahme des Spek-

trums der 0,01 norm. $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})_3]\text{Cl}_2$ -Lösung in 0,1 norm. NH_4OH zu machen (siehe Lösung T oben).

Eine ammoniakalische Lösung von $[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})\text{Cl}]\text{Cl}_2$ bzw. $[\text{Co}(\text{NH}_3)_4\text{Cl}_2]\text{Cl}^{7)}$ gibt identische Absorption mit der von

7) Beide 1, 2- und -1, 6-Dichlorosalze gehen in Lösung in dasselbe Diaquosalz über.

$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]\text{Cl}_3$. Ebenso verhalten sich in dieser Beziehung gleich ammoniakalische Lösungen von $[\text{Co}(\text{NH}_3)_3(\text{H}_2\text{O})\text{Cl}_2]\text{Cl}$ und $[\text{Co}(\text{NH}_3)_2(\text{H}_2\text{O})_2]\text{Cl}_3$, wie es bei den ammoniakalischen Lösungen von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und $[\text{Co}(\text{NH}_3)_6]\text{Cl}_2$ der Fall ist (vgl. S. 5).

Aus allen oben angegebenen Tatsachen kann man folgern, dass Aquokobaltiammine im allgemeinen mit Aminen in der Lösung eine Reihe neuer Ammine geben. Diese Ammine haben ähnliche Konstitution, gleichgültig, ob sie aus Mono-, Di- oder Triaquosalzen gebildet werden. Die Komplexradikale kann man demnach folgendermassen schreiben :

$[(\text{NH}_3)_5\text{Co}(\text{OX})]$ aus Monoaquopentamminsalz, $[(\text{NH}_3)_4\text{Co}(\text{OX})_2]$ aus Diaquotetramminsalz und $[(\text{NH}_3)_3\text{Co}(\text{OX})_3]$ aus Triaquotriamminsalz.

Im Folgenden seien nun alle denkbaren Reaktionsweisen zwischen Aquokobaltiamminen und Aminen näher erörtert, um schliesslich festzustellen, dass folgendes die einzige Erklärung für die angeführten Beobachtungen sein muss (vgl. S. 4):

Aquokobaltiammin + Amin \rightleftharpoons unbekanntes neues Ammin.

1) Wenn die Gleichung (2) auf S. 3, die sich auf die Reaktion zwischen $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und NH_4OH bezieht, richtig wäre, müsste sich NH_4Cl bilden. Kann nun der Unterschied der Absorption der wässrigen Lösung des $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ einerseits und der ammoniakalischen Lösung des $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ andererseits durch das nach obiger Gleichung gebildete NH_4Cl bedingt sein? Es ist von vornherein sehr unwahrscheinlich, dass NH_4Cl einen solchen Einfluss haben könnte. Sicherheitshalber aber haben wir ein Absorptionsspektrum der gemischten Lösung von $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ und NH_4Cl aufgenommen. Die Lösung zeigte nur dieselbe Absorption wie eine $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ -Lösung und eine ganz andere als die der ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$. Das heisst, bei der Reaktion zwischen Aquokobaltiamminen und Aminen bilden sich keine Hydroxosalze und die Gleichung (2) entspricht nicht dem wirklichen Reaktionsverlauf.

2) Aus $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und NH_4OH kann sich $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ bilden, aber, wie allgemein bekannt, geht diese Reaktion bei

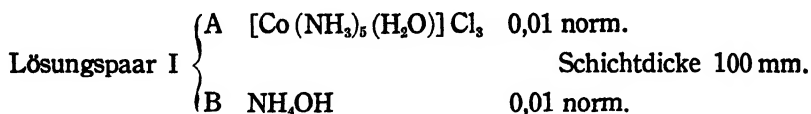
Zimmertemperatur und bei solch einer kleinen Konzentration nicht vor sich. Zudem ist die Farbe dieser ammoniakalischen Lösung auch verschieden von der des $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$. Ueberdies kann man sich noch durch folgendes davon überzeugen, dass sich $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ dabei nicht bildet: Fügt man nämlich zur ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ in der Kälte HCl , so entsteht $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ nicht hingegen $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ wieder. Ferner kann man keine denkbare Gleichung für die Reaktion zwischen $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und $(\text{CH}_3)_4\text{NOH}$ angeben, um damit zu einem Salz vom Hexammin-typus zu kommen, obgleich $(\text{CH}_3)_4\text{NOH}$ genau wie NH_4OH reagiert (siehe S. 6). Wenn sich überdies $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ aus $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und NH_4OH in solch einer verdünnten Lösung bilden würde, sollten sowohl $[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]\text{Cl}_3$ als auch $[\text{Co}(\text{NH}_3)_3(\text{H}_2\text{O})_3]\text{Cl}_3$ dasselbe Salz $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ geben. Aber das ist nicht der Fall, wie Absorptionskurven zeigen (siehe Fig. 6 und 7). Mithin kommt die Bildung von $[\text{Co}(\text{NH}_3)_6]\text{-Salz}$ nicht in Frage.

3) Es wäre weiterhin denkbar, dass ein Teil des $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ unter der Wirkung von NH_4OH in $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ übergehen könnte. Die ammoniakalische Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ müsste demnach $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ und $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ enthalten. Wenn dies der Fall wäre, sollte sich mehr $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ bilden, falls die NH_4OH -Konzentration grösser wird. Aber wie schon in Fig. 2 gezeigt, nähert sich die Absorptionskurve der ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ der von $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ nicht. Dass in der Lösung noch viel $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ unangegriffen bleibt, kann auch nicht angenommen werden, da auch ein schwaches Alkali wie Na_2CO_3 schon praktisch vollständig $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$ aus $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ gibt (siehe Fig. 1), usw. usw. Als die einzige richtige Erklärung bleibt demnach die oben angeführte übrig.

Ermittlung des Reaktionsverhältnisses zwischen Aquokobaltiammin und Amin in der Lösung mittels Spektrographischer Methode.

Wir haben oben mitgeteilt, dass Aquokobaltiammin mit Amin im allgemeinen in der Lösung unter Bildung einer neuen Art Ammin

an der Stelle des koordinativen Wassers reagiert. Um nun das Reaktionsverhältnis beider Verbindungen festzustellen, haben wir zunächst die von SHIBATA angegebene spektrographische Methode⁸⁾ gebraucht. An Hand von folgendem Lösungspaar sei die Methode kurz erklärt:



Man stellt zunächst z. B. folgende Reihe der gemischten Lösungen her:

- | | |
|------------------------------|-------------------------|
| 1) Lösung A (kein B dabei) | 2) 5 Vol. A + 1 Vol. B |
| 3) 4 Vol. A + 1 Vol. B | 4) 3 Vol. A + 1 Vol. B |
| 5) 7 Vol. A + 3 Vol. B | 6) 2 Vol. A + 1 Vol. B |
| 7) 3 Vol. A + 2 Vol. B | 8) 1 Vol. A + 1 Vol. B |
| 9) 2 Vol. A + 3 Vol. B | 10) 1 Vol. A + 2 Vol. B |
| 11) 3 Vol. A + 7 Vol. B | 12) 1 Vol. A + 3 Vol. B |
| 13) 1 Vol. A + 4 Vol. B | 14) 1 Vol. A + 5 Vol. B |
| 15) Lösung B (kein A dabei). | |

Nun photographiert man die Absorptionsspektren dieser gemischten Lösungen eins nach dem andern nebeneinander auf einer Platte, wobei die Schichtdicke und alle anderen Bedingungen stets konstant gehalten werden.

Wie aus dem oben angegebenen zu ersehen ist (siehe Fig. 2 und 4), verschiebt sich die Endabsorption vom betreffenden Ammin nach Rot, und deshalb muss die Konzentration des betreffenden Ammins am grössten sein in derjenigen Lösung, welche das Maximum der Endabsorption gibt. Da ferner alle oben angegebenen Lösungsgemische identische Gesamtkonzentration haben, d. h. alle 0,01 norm. sind, muss die Konzentration des entstehenden Ammins am grössten sein in derjenigen gemischten Lösung, in welcher das Volumverhältnis der beiden Urlösungen A und B dasselbe wie das Reaktionsverhältnis der beiden Substanzen ist. Diese Beziehung kann sehr leicht mathe-

8) Y. SHIBATA, T. INOUE u. Y. NAKATSUKA, Japan. Journ. Chem. 1, 1 (1922).

matisch hergeleitet werden⁹⁾. Fig 8 zeigt das Resultat, das mit dem oben angegebenen Lösungspaar I erhalten wurde. Als Abszisse ist das Volumverhältnis beider Lösungen A und B, und als Ordinate die Wellenlänge des Absorptionsendes in Å. E. aufgetragen. Die Schichtdicke der einzelnen gemischten Lösung betrug immer 100 mm. Das Maximum der Endabsorption liegt bei der gemischten Lösung (1 Vol. A + 1 Vol. B) d. h. ein Mol. $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ reagiert mit einem Mol. NH_4OH .

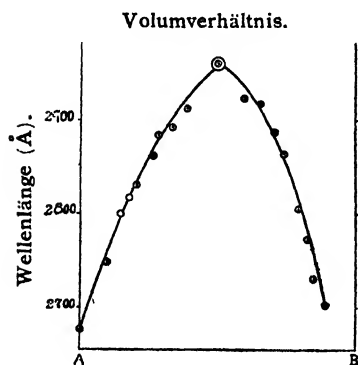


Fig. 8.

Lösungspaar I.

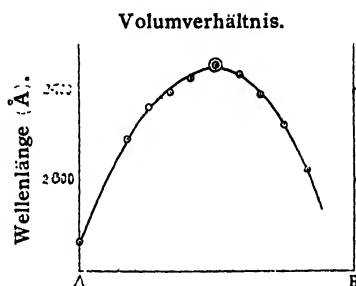


Fig. 9.

Lösungspaar II.

Ein noch konzentrierteres Lösungspaar gibt dasselbe Resultat, wie in Fig. 9 für nachstehendes angegeben :

Lösungspaar II	{	A	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$	0,1 norm.	Schichtdicke 10 mm.
		B	NH_4OH	0,1 norm.	

Mit dem Sulfat erhält man gleichfalls eine Kurve, die bei der gemischten Lösung 1 : 1 das Maximum hat (vgl. Fig. 10):

Lösungspaar III	{	A	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$	0,01 norm.	Schichtdicke 100 mm.
		B	NH_4OH	0,01 norm.	

Andere Amine als NH_3 reagieren ebenso wie dieses. In Fig. 11 sind die Resultate angegeben, die mit folgenden Lösungspaaen erhalten wurden :

9) T. INOUE, *Japan Journ. Chem.* 3, 132 (1928)

Lösungspaar IV	{	A	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})_2](\text{SO}_4)_3$	0,01 norm.	Schichtdicke 100 mm.
		B	$\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$	0,02 norm.	
Lösungspaar V	{	A	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})_2](\text{SO}_4)_3$	0,01 norm.	Schichtdicke 100 mm.
		B	$(\text{C}_2\text{H}_5)_2\text{NH}$	0,01 norm.	

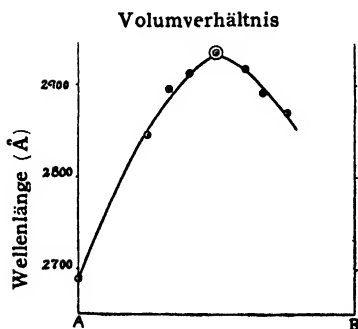


Fig. 10.
Lösungspaar III.

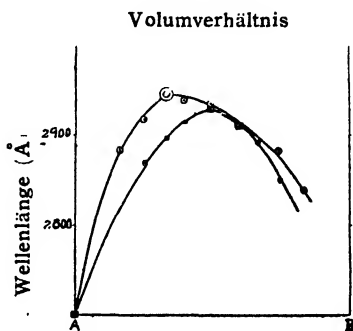


Fig. 11.
—○—○— Lösungspaar IV.
—□—□— Lösungspaar V.

Beim Lösungspaar IV findet man das Maximum an der Stelle, an der das Volumverhältnis 2 : 1 ist. Dies ist deswegen selbstverständlich, da die Konzentration des Amins als Base hier doppelt so gross ist wie in den anderen Fällen.

Ferner wurden noch folgende Lösungspaare untersucht :

Lösungspaar VI	{	A	$[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2](\text{SO}_4)_3$	0,01 norm.	Schichtdicke 100 mm.
		B	$\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$	0,02 norm.	
Lösungspaar VII	{	A	$[\text{Co en}_2(\text{H}_2\text{O})_2]\text{Cl}_3$	0,01 norm.	Schichtdicke 100 mm.
		B	$\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$	0,02 norm.	

Fig. 12 und 13 geben die mit diesen beiden Lösungspaaren erhaltenen Ergebnisse an.

Die ammoniakalische Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})_3]\text{Cl}_3$ ist sehr unbeständig und trübt sich sogleich nach der Herstellung. Daher kann man mittels dieser Methode das Reaktionsverhältnis nicht ermitteln, aber infolge der Regelmässigkeit der Absorptionskurven der

aminhaltigen Lösungen der Mono-, Di- und Triaquokobaltiammine, die schon auf S. 8 beschrieben wurde, kann man ohne Zweifel schliessen, dass das Triaquoradikal auch ganz ähnlich reagieren wird.

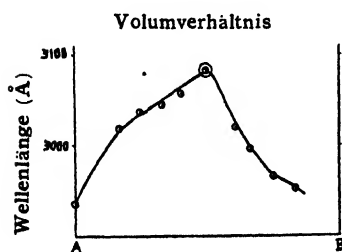


Fig. 12.
Lösungspaar VI.

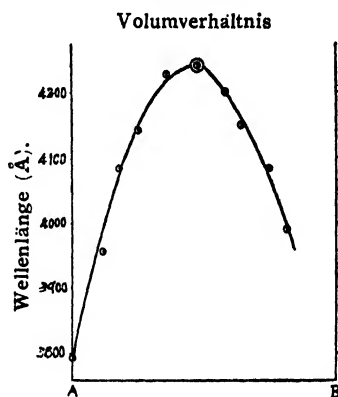


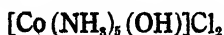
Fig. 13.
Lösungspaar VII.

Alle bis eben beschriebenen Tatsachen zeigen also folgendes: Aquokobaltiammine reagieren mit Ammoniak bzw. Aminen im allgemeinen in der Lösung unter Bildung einer neuen Reihe Ammine, und zwar in solchem Verhältnis, dass auf ein koordinatives Wassermolekül ein basisches Stickstoffatom von Ammoniak bzw. Amin kommt!

Zum Vergleich sind die Resultate von folgenden Systemen in Fig. 14 und 15 angegeben:

Lösungspaar VIII	{	A $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$	0,01 norm.
			Schichtdicke 100 mm.
		B NH_3	0,01 norm.
Lösungspaar IX	{	A $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_2$	0,01 norm.
			Schichtdicke 100 mm.
		B Na_2CO_3	0,01 norm.

Das Lösungspaar VIII gibt natürlich kein Maximum, wogegen IX ein deutliches Maximum zeigt, da sich in diesem Falle



bildet (vgl. Fig. 1).

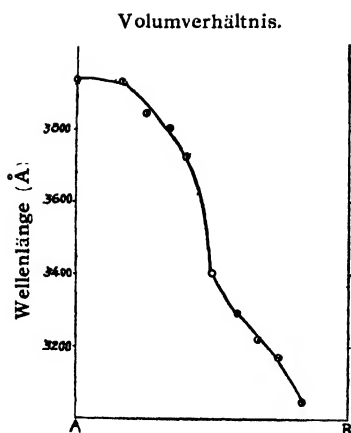


Fig. 14.
Lösungspaar VIII.

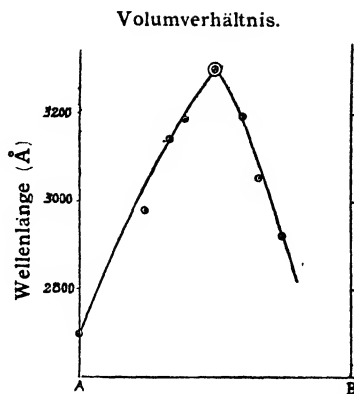


Fig. 15.
Lösungspaar IX.

Ermittlung des Reaktionsverhältnisses mittels Viskositätsmessungen.

Die Viskosität ist bekanntlich keine einfach festzulegende Grösse, insbesondere wenn es sich um Elektrolytlösungen handelt. Im allgemeinen verlaufen die Viskositäts-Konzentrations-Kurven von Elektrolyten konvex gegen die Konzentrationachse; falls man aber den Konzentrationsbereich zwischen 0-0,1 norm. wählt, können die Kurven als gerade Stück betrachtet werden¹⁰⁾. Wenn deshalb A und B in der Lösung nicht miteinander reagieren, oder beide keinen merklichen Einfluss aufeinander bzw. auf ihren molekularen Zustand haben, dann sind die Beziehungen zwischen Viskosität und Konzentration wie in Fig. 16 angegeben. Die gerade Linie CB zeigt die von A, DA die von B, und CD die von (A + B). Wir haben diese Beziehung mit folgenden Lösungsparen geprüft, bei denen A und B nicht miteinander reagieren :

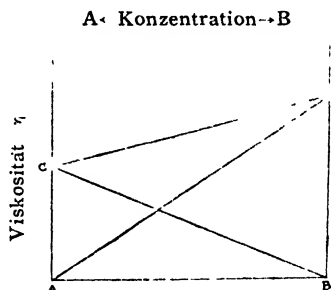
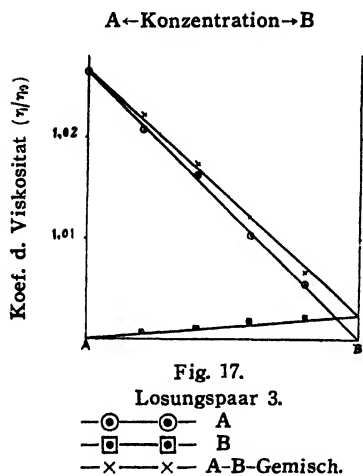


Fig. 16.

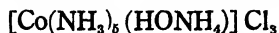
10, Vgl. LANDOLT-BOERNSTEIN, Physikalisch-chemische Tabellen.

Lösungspaar 1	{	A	$[\text{Co}(\text{NH}_3)_6] \text{Cl}_3$	0,1 norm.	Temperatur 25,0°
		B	NH_3	0,1 norm.	
Lösungspaar 2	{	A	$[\text{Co}(\text{NH}_3)_6(\text{H}_2\text{O})_2] (\text{SO}_4)_3$	0,1 norm.	Temperatur 25,0°
		B	$\text{H}_2\text{N}\cdot\text{COOC}_2\text{H}_5$	0,1 norm.	
Lösungspaar 3	{	A	$[\text{Co}(\text{NH}_3)_6(\text{H}_2\text{O})] \text{Cl}_3$	0,1 norm.	Temperatur 25,0°
		B	$\text{CO}(\text{NH}_2)_2$	0,1 norm.	

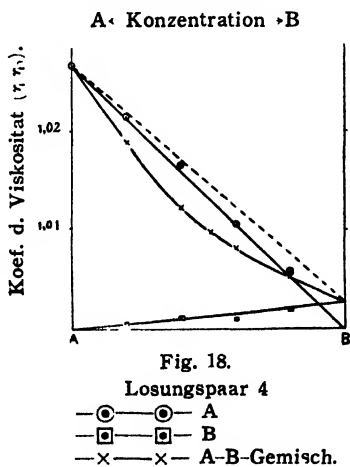


Das mit dem Lösungspaar 3 erhaltene Ergebnis ist in Fig. 17 dargestellt.

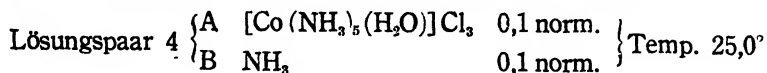
Nun reagiert aber beispielsweise $[\text{Co}(\text{NH}_3)_6(\text{H}_2\text{O})] \text{Cl}_3$ in Lösung mit NH_3 , weshalb die Viskositätskurve dieses Lösungspaares von der oben angegebenen Linie CD abweichen kann. Als Reaktionsprodukt von $[\text{Co}(\text{NH}_3)_6(\text{H}_2\text{O})] \text{Cl}_3$ und NH_3 in der Lösung kann ferner sehr wahrscheinlich



angesehen werden (vgl. S. 12 f). Wenn aber dieses Ammin gebildet wird, nimmt die gesamte Zahl der Moleküle in der gemischten Lösung ab. Die Viskosität der in verschiedenem Verhältnis gemischten Lösung kann daher vielleicht etwas kleiner sein als die Linie CD zeigt. Und wenn es überhaupt eine maximale Abweichung der Viskosität gibt, werden $[\text{Co}(\text{NH}_3)_6(\text{H}_2\text{O})] \text{Cl}_3$ und NH_3 in demjenigen Verhältnis reagieren, welches



der Stelle der maximalen Abweichung entspricht. Dieses geht bei diesem Lösungspaar wirklich vor sich, wie Fig. 18 zeigt:



Das Abweichungsmaximum liegt an der Stelle, an der das Volumverhältnis beider Lösungen 1:1 ist, also ein Mol. $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Cl}_3$ reagiert mit einem Mol. NH_3 in der Lösung. Dieses Ergebnis stimmt mit dem durch die spektrographische Methode erhaltenen vollkommen überein.

Wir haben auch die Viskosität des folgenden Lösungspaares gemessen:

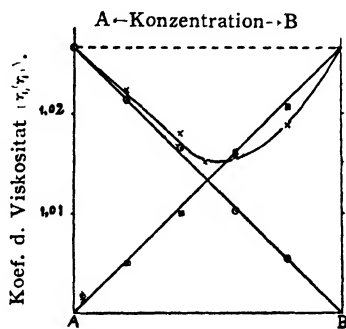
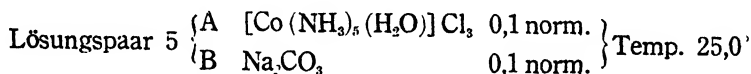


Fig. 19.

Lösungspaar 5

- ○ - - - A
 - □ - - - B
 - x - - - A-B Gemisch.

Auch dieses Paar gibt das Abweichungsmaximum beim Verhältnis 1:1 (siehe Fig. 19), aber das Reaktionsprodukt ist in diesem Falle das Salz $[\text{Co}(\text{NH}_3)_5(\text{OH})]\text{Cl}_2$, wie schon auf S. 2 beschrieben worden.

Gefrierpunktserniedrigung der gemischten Lösungen von Aquopentamminkobaltisalzen und Ammoniak bzw. Aethylendiamin.

Wie schon auf S. 16 beschrieben wurde, besteht in der ammoniakalischen Lösung von $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz mit grösster Wahrscheinlichkeit folgendes Gleichgewicht:



Wir haben ferner schon festgestellt, dass Gleichgewicht sich weitgehend nach rechts verschiebt, wenn man die Lösung mit Eis erkalten lässt (vgl. S. 4). Wenn nun $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{X}_3$ und $[\text{Co}(\text{NH}_3)_5(\text{HONH}_2)]\text{X}_3$

in der verdünnten Lösung vollständig oder gleichermassen ionisieren, und die oben genannte Reaktion zu Ende verläuft, nimmt die gesamte Zahl von Molekülen bzw. Ionen ab. Daher muss die Gefrierpunkts-erniedrigung einer solchen Lösung in demjenigen Masse kleiner werden wie die Summe der Erniedrigungen der beiden Lösungen, $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salz und NH_3 , was sich aus der Molekularniedrigung berechnen lässt. Wir haben zuerst das System $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3-\text{NH}_3$ studiert. Die Gefrierpunktserniedrigungen sind folgende:

$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ -Lösung 0,02 norm. (0,01 molar) 0,076°.....(1)

NH_3 -Lösung 0,02 norm. 0,044°.....(2)

Gemischte Lösung, worin die Konzentration vom

Ammin und NH_3 dieselbe wie oben 0,088°.....(3).

Vergleicht man die Summe (1)+(2) d. h. 0,120°, mit (3), so findet man (3) viel kleiner und zwar in dem Masse, dass die Differenz (0,032°) fast genau der theoretischen Erniedrigung der 0,02 molaren wässrigen Lösung (0,034°) entspricht. Man kann daher sagen, dass die oben angegebene Reaktion bei 0° fast vollständig nach rechts abläuft, und der grösste Teil des Ammoniaks in der Lösung als $[\text{Co}(\text{NH}_3)_5(\text{HONH}_4)]$ -Salz vorliegt.

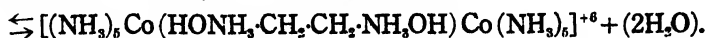
Wir haben weiter Aethylendiamin statt Ammoniak angewandt, wobei sich folgende Erniedrigungswerte ergaben:

$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ -Lösung wie oben im Versuch mit Ammoniak

$\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ -Lösung 0,02 norm. (0,01 molar) 0,019°

Gemischte Lösung; die Konzentration der beiden gelösten Stoffe wie oben mit Ammoniak 0,073°.

In diesem Falle ist die Reaktionsgleichung wie folgt:



Es bildet sich also hier ein sechswertiges positives Radikal. Dieses Radikal ist von ganz besonderem Typus; die beiden Kobaltatome sind durch Aethylendiamin als Brückenglied mittels Nebenvalenzbindung verknüpft.

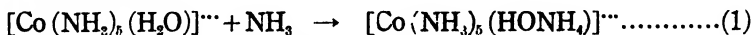
Wenn $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]_2(\text{SO}_4)_3$ und $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ gänzlich

miteinander reagierten, würde die Erniedrigung der gemischten Lösung noch viel kleiner sein als die beobachtete. Die gefundene, etwas grössere Erniedrigung als die theoretische, ist der Unvollkommenheit der Reaktion zuzuschreiben, d. h. das oben angegebene Gleichgewicht verschiebt sich nicht genug nach rechts. Die Bildung des $[\text{Co}(\text{NH}_3)_5(\text{HONH}_2)]$ -Radikals folgt nach dem Zusammentreffen eines $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Radikals mit einem Molekül NH_3 ; dagegen bildet sich das $[(\text{H}_3\text{N})_6\text{Co}(\text{HONH}_2\text{CH}_2\text{CH}_2\text{NH}_2\text{OH})\text{Co}(\text{NH}_3)_5]$ -Radikal aus zwei $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Radikalen und einem Molekül $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$. Es ist aber einzusehen, dass das erstere Zusammentreffen viel häufiger vor sich gehen kann, und daher ist es sehr natürlich, dass die letztere Reaktion unvollkommener ist als die erstere.

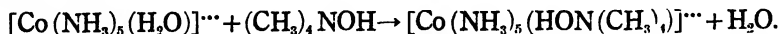
Zusammenfassend kann man also feststellen: In einer gemischten Lösung von Aquokobaltiammin und Ammoniak bzw. Amin entsteht eine neue Art Ammin, welches quartäres Ammoniumhydroxyd anstelle eines Wassermoleküls des Mutteraquoammins am Kobaltatom koordiniert enthält.

Der Mechanismus der Bildung des neuen Ammins.

Für die Bildung von $[\text{Co}(\text{NH}_3)_5(\text{HONH}_2)]$ -Salzen aus $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]$ -Salzen und NH_3 in Lösung können folgende zwei Reaktionsweisen angegeben werden:



Wie schon auf S. 6 gezeigt, reagiert $(\text{CH}_3)_4\text{NOH}$ ganz genau wie NH_3 , sodass für diesen Fall nur die Gleichung vom Typus (2) gültig sein kann, also:

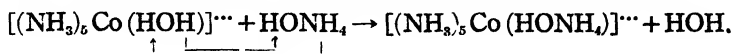


Deshalb ist es viel wahrscheinlicher, dass Amine sich nicht als solche mit Aquokobaltiamminen vereinigen, sondern im allgemeinen als quartäre Ammoniumbasen unter Abspaltung von Wasser reagieren.

Diese Reaktionsweise wird auch einigermaßen durch folgende Tatsache gestützt: Pyridin, eine schwächere Base als Ammoniak

bzw. Alkylamine, reagiert viel unvollkommener, wie schon auf S. 7 gezeigt. Da die Stärke des Amins als Base in erster Linie vom Wasseradditionsvermögen abhängig ist, und Pyridin Pyridiniumhydroxyd in viel geringerem Masse als Ammoniak Ammoniumhydroxyd bildet, reagiert Pyridin viel schwächer.

Der Mechanismus der Bildung muss also wie folgt sein:



Zusammenfassung.

1) Aus dem Absorptionsspektrum wurde gefunden, dass Aquokobaltiammin und Ammoniak bzw. Amin in Lösung ein entsprechendes Hydroxosalz nicht bilden, sondern eine neue Art Ammin.

2) Dass in der ammoniak- bzw. aminhaltigen Lösung von Aquokobaltiammin sich kein Hydroxosalz bildet, wurde auch durch die Gefrierpunktniedrigung solcher Lösungen bewiesen.

3) Spektrographische Untersuchungen und auch Viskositätsmessungen zeigten, dass Aquokobaltiammin mit Ammoniak bzw. Amin in solchem Verhältnis reagiert, dass einem koordinativen Wassermolekül ein basisches Stickstoffatom entspricht.

4) Die neue Art Ammin enthält quartäres Ammoniumhydroxyd an derjenigen Koordinationsstelle gebunden, welche vom Wassermolekül des Mutteraquokobaltiammins besetzt war.

Die meisten Teile der spektrographischen Arbeit wurden 1924 im Laboratorium von Prof. Y. SHIBATA der Universität Tokyo durchgeführt.

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Ueber Flächen und Kurven (VI): Eiliniën und Eiflächen

Sôji MATSUMURA

(Accepted for publication, September 28, 1933)

In dieser Arbeit möchte ich über Eiliniën und Eiflächen einige Bemerkungen machen.

- (1) Zu meiner Arbeit „*Ueber konvex-geschlossene Flächen*“ in Tôhoku Math. Journ. 36, p. 192.

(1)

Man setze $\tau = R_1 R_2$.

Dann lautet (2)

$$(a) \quad \bar{x} - x = \tau \bar{y} - \tau y.$$

Wegen $(x_1, x_2, \bar{x}_i) = 0$ ist

$$(b) \quad x = -\lambda \bar{x} = (R_1 R_2)^{\frac{1}{2}} \bar{x} = \tau^{\frac{1}{2}} \bar{x}$$

und bei der Affinentfernung ist

$$(c) \quad P = (\bar{x} - x) x = \tau + \lambda \bar{\tau} = (p + \bar{p}) \tau^{\frac{1}{2}},$$

wobei p, \bar{p} Stützabstände sind.

Nach (b) ist

$$x = \tau^{\frac{1}{2}} \bar{x} = -\lambda \bar{x} = -\lambda \tau^{-\frac{1}{2}} \bar{x}$$

wegen $\bar{x} = -\bar{x}$ ist

$$(d) \quad \lambda \tau^{\frac{1}{2}} = \bar{\tau}^{\frac{1}{2}}.$$

Dann ist nach (c)

$$\tau + \tau^{\frac{1}{2}} \cdot \bar{\tau}^{\frac{3}{2}} = (p + \bar{p}) \tau^{\frac{1}{2}},$$

d.h.

$$(e) \quad \tau^{\frac{3}{2}} + \bar{\tau}^{\frac{3}{2}} = p + \bar{p} = b \quad (= \text{Breite}).$$

Aus (a) folgt

$$\bar{x}_i - x_i = \tau_i \eta - \bar{\tau}_i \bar{\eta} + \dots x_i + \dots \bar{x}_i,$$

bei Multiplikation mit x ist nach (b)

$$(f) \quad \tau_i + \lambda \bar{\tau}_i = 0,$$

d.h. wegen (d) und (e) ist

$$(g) \quad b_i = \frac{1}{4} \left(-\frac{\tau_i}{\tau^{\frac{1}{2}}} + -\frac{\bar{\tau}_i}{\bar{\tau}^{\frac{1}{2}}} \right) = 0,$$

also ist die Eifläche von konstanter Breite.

Dann ist aber

$$(h) \quad b = R_1 + R_1 = R_2 + R_2,$$

$$(k) \quad b = \frac{R_1 + R_2}{2} + \frac{R_1 + \bar{R}_2}{2} \geq \sqrt{R_1 R_2} + \sqrt{\bar{R}_1 \bar{R}_2}.$$

Nach (e) ist

$$b = \tau^{\frac{3}{2}} + \bar{\tau}^{\frac{3}{2}} \geq \tau^{\frac{1}{2}} + \bar{\tau}^{\frac{1}{2}}.$$

Nach (h) aber wird

$$\tau = R_1 R_2 = b^2 - b(R_1 + \bar{R}_2) + \bar{\tau},$$

also

$$\begin{aligned} \tau - \bar{\tau} &= b^2 - b(\bar{R}_1 + \bar{R}_2) \\ &= (\tau^{\frac{1}{2}} + \bar{\tau}^{\frac{1}{2}}) (\tau^{\frac{1}{2}} - \bar{\tau}^{\frac{1}{2}}) \leq b (\tau^{\frac{1}{2}} - \bar{\tau}^{\frac{1}{2}}), \end{aligned}$$

also

$$b - (\bar{R}_1 + \bar{R}_2) \leq \tau^{\frac{1}{2}} - \tau^{-\frac{1}{2}},$$

analog

$$b - (R_1 + R_2) \leq \tau^{-\frac{1}{2}} - \tau^{\frac{1}{2}},$$

zusammen :

$$2b - (R_1 + R_2) - (\bar{R}_1 + \bar{R}_2) \leq 0.$$

Nach (k) muss also hier und überall vorher das Gleichheitszeichen stehen, also auch in (k), d.h. $R_1 \equiv R_2$, d.h. alle Punkte sind Nabelpunkte, also ist die Fläche eine Kugel,⁽¹⁾ w.z.b.w.

Auf dieselbe Weise erfolgt der folgende Satz :

Die Kugeln sind die einzigen Eiflächen mit dem Mittelpunkt O, für die die Punkte

$$p = \xi + H^2 \eta$$

oder

$$p = \xi + \frac{1}{2} \left(-\frac{1}{R_1} + \frac{1}{R_2} \right) \eta$$

oder u.s.w.

in Gegenpunkten zusammenfallen.⁽²⁾

(2)

Nach Affingeometrie haben wir⁽³⁾

$$(1) \quad |\eta, \xi_1, \xi_2| = |G|^{\frac{1}{2}}.$$

Aus⁽⁴⁾

(1) Vergl. BLASCHKE, W.: Vorlesungen über Differentialgeometrie II, Berlin (1923) § 65.

(2) Vergl. MATSUMURA, S.: Ueber Flächen und Kurven (III), Mem. of the Fac. of Sci. and Agri., Taihoku Imp. Univ., Formosa, Japan, Vol. V (1933) p. 300.

(3) KAWAGUCHI, A.: Differentialgeometrie 3, Zoku Bankin Kotosugakoza IIE, Kyoritsusha, p. 150.

(4) MATSUMURA, S.: Über konvex-geschlossene Flächen, Tôhoku Math. Journ. Vol. 36 (1933) p. 192.

$$(2) \quad p = \xi + (R_1 R_2) \eta$$

ergibt sich also

$$(3) \quad |p, \xi_1, \xi_2| - |\xi, \xi_1, \xi_2| = |G|^{\frac{1}{2}} (R_1 R_2).$$

Nach Affingeometrie ergibt sich⁽⁵⁾

$$(4) \quad \xi_{\lambda\mu} = a_{\lambda\mu}^{\ddot{\nu}} \xi_{\nu} + g_{\lambda\mu} \eta,$$

aus (1) und (4) ergibt sich also

$$|\xi_{\lambda\mu} - a_{\lambda\mu}^{\ddot{\nu}} \xi_{\nu}, \xi_1, \xi_2| = G,$$

d.h.

$$(5) \quad |\xi_{\lambda\mu}, \xi_1, \xi_2| - a_{\lambda\mu}^{\ddot{\nu}} |\xi_{\nu}, \xi_1, \xi_2| = G,$$

d.h.

$$(6) \quad |\xi_{\lambda\mu}, \xi_1, \xi_2| = G.$$

Wir haben in dem Falle Affinminimalflächen⁽⁶⁾

$$z = (\xi_1 \xi_2 \xi) \lambda^{\frac{1}{2}} \delta \lambda \div F$$

oder

$$(7) \quad \frac{zF}{\lambda^{\frac{1}{2}} \delta \lambda} = (\xi_1 \xi_2 \xi).$$

Aus (3), (7) erfolgt

$$(8) \quad (\xi_1 \xi_2 p) = (R_1 R_2) |G|^{\frac{1}{2}} + zF \div (\lambda^{\frac{1}{2}} \delta \lambda),$$

d.h.

$$(9) \quad z = \frac{[(\xi_1 \xi_2 p) - (R_1 R_2) |G|^{\frac{1}{2}}] \lambda^{\frac{1}{2}} \delta \lambda}{F}.$$

Aus (3) folgt nun

$$(10) \quad \frac{1}{3} \int \int (\xi_1 \xi_2 p) du^1 du^2 - \frac{1}{3} \int \int (\xi_1 \xi_2 \xi) du^1 du^2$$

(5) l. c. (3) p. 157.

(6) BLASCHKE, W.: Differentialgeometrie (II), S. 181.

$$= -\frac{1}{3} \iint \{R_1 R_2\} |G|^{\frac{1}{2}} du^1 du^2.$$

Für die Eifläche mit H konst aus (10) folgt⁽¹⁾

$$(11) \quad -\frac{1}{3} \iint (\xi_1 \xi_2 p) du^1 du^2 = -\frac{1}{3} \iint \{R_1 R_2\} |G|^{\frac{1}{2}} du^1 du^2 + V_{..}$$

Die Relativoberfläche der Fläche (ξ) bezüglich der Eifläche (p) ist mit

$$(12) \quad \iint \{R_1 R_2\} |G|^{\frac{1}{2}} du^1 du^2 + 3 V$$

gegeben.⁽²⁾

Wenn

$$R_1 R_2 = \text{const.}$$

ist, dann folgt aus (12)

$$(13) \quad \{R_1 R_2\} \iint |G|^{\frac{1}{2}} du^1 du^2 + 3 V.$$

(2) Ueber zwei Flächen, die eine Beziehung haben

(1)

Wir betrachten

$$(I) \quad \sigma \theta_u + \theta_v + \lambda \theta_w = 0.$$

Ist $\hat{\xi}$ ein Kreis im R_2 und \mathfrak{z} ein nicht auf ihm gelegener Punkt, so ist

$$(1) \quad \bar{\xi} = 2(\xi \hat{\xi}) \hat{\xi} - \xi$$

der zu \mathfrak{z} in bezug auf den Kreis $\hat{\xi}$ inverse Punkt.

Aus (1) folgt

(1) I. c. (3), S. 201.

(2) I. c. (3), S. 205.

Oder BLASCHKE, W.: Vorlesungen über Differential-Geometrie II, S. 181.

$$(2) \quad \begin{cases} \bar{x}_u = 2(x_u \hat{\xi}) \hat{\xi} - x_u, \\ \bar{x}_v = 2(x_v \hat{\xi}) \hat{\xi} - x_v, \\ \bar{x}_{uv} = 2(x_{uv} \hat{\xi}) \hat{\xi} - x_{uv}, \end{cases}$$

so ergibt sich

$$(3) \quad \begin{aligned} & (\lambda \bar{x}_{uv} + \sigma \bar{x}_u + \bar{x}_v) \\ &= (\lambda x_{uv} + \sigma x_u + x_v, \hat{\xi}) \hat{\xi} - (\lambda x_{uv} + \sigma x_u + x_v). \end{aligned}$$

Aus (3) wissen wir, dass, wenn \mathfrak{g} (I) erfüllt ist, $\bar{\mathfrak{g}}$ auch dann (I) erfüllt sein muß.

Aus (2₁) ergibt sich

$$(\bar{x}_u \bar{x}_v) = 4(x_u \hat{\xi})(x_v \hat{\xi}) + (x_u x_v) - 4(x_u \hat{\xi})(x_v \hat{\xi}),$$

d.h.

$$(\bar{x}_u \bar{x}_v) = (x_u x_v),$$

also folgt

$$\bar{E} = E,$$

wobei $(\hat{\xi} \hat{\xi}) = 1$ ist.

Auf dieselbe Methode kann man beweisen:

$$\bar{F} = F,$$

$$\bar{G} = G,$$

also

$$(4) \quad \begin{cases} \bar{E} = E, \\ \bar{F} = F, \\ \bar{G} = G, \end{cases}$$

und daher ist λ, σ in (I) invariant gegen jede beliebige Inversion. Auch kann man beweisen⁽¹⁾

(1) ROTHE, R.: Ueber die Inversion einer Fläche und konforme Abbildung zweier Flächen aufeinander mit Erhaltung der Krümmungslinien, Math. Ann. Bd. 72 (1912) S. 60.

$$(5) \quad \frac{\bar{L}-L}{E} = \frac{\bar{M}-M}{F} = \frac{\bar{N}-N}{G}.$$

Also gilt es in unserem Falle nach⁽¹⁾ (7):

$$(6) \quad \frac{\bar{L}-L}{E} = \frac{M}{F} = \frac{\bar{N}-N}{G}.$$

(2)

Hier möchte ich zwei Flächen $\xi(u, v)$ und $\bar{\xi}(u, v)$ studieren, für welche das Strahlensystem, welches von den gemeinschaftlichen Tangenten dieser Flächen gebildet wird, die RIBAUCCOURSCHE Kongruenz ist. Daraus entstehen folgende Beziehungen

$$(1) \quad \xi_u + \frac{\sigma}{\lambda} \xi_v + \frac{1}{\lambda} \xi = 0,$$

wobei

$$\frac{\sigma}{\lambda} = U_1 V'_1, \quad \frac{1}{\lambda} = U'_1 V_1,$$

U, V Funktionen von u bzw. v allein sind.

Wenn

$$\frac{\partial \left(\frac{\sigma}{\lambda} \right)}{\partial u} = \frac{\partial \left(-\frac{1}{\lambda} \right)}{\partial v} \quad (= U'_1 V_1)$$

ist, kann man (1) ausdrücken in der Form:⁽²⁾

$$(2) \quad \frac{\partial^2 \xi}{\partial u \partial v} = 0.$$

Also folgt aus (2)

$$\xi = f(u) + \varphi(v),$$

d.h., unsere Fläche ist eine Translationsfläche.

(1) NAKAJIMA, S.: Ueber zwei Flächen, welche eine Beziehung haben, I, Tôhoku Math. Journ. Vol. 30 (1928) p. 143.

(2) EISENHART, L. P.: Transformations of planar Nets, American J. of Math. XL (1918) p. 131.

(3)

Wir betrachten hier affine Abbildung $\xi(u, v) \rightarrow \bar{\xi}(u, v)$, dann hat man aus (I) in §1 das Ergebnis:

$$(1) \quad \text{const. } \{\bar{\lambda} \xi_{uv} + \bar{\sigma} \xi_u + \xi_v\} + \text{const. } (\bar{\lambda} + \bar{\sigma} + 1) = 0.$$

Für jede Wahl von gemeinsamen Flächenparametern (u, v) seien die ersten Fundamentalgrößen in zugeordneten Punkten P einander gleich.

$$\text{d. h.} \quad E = \bar{E}, \quad F = \bar{F}, \quad G = \bar{G},$$

dann folgt aus (1)

$$(2) \quad \text{const. } \{\lambda \xi_{uv} + \sigma \xi_u + \xi_v\} + \text{const. } (\lambda + \sigma + 1) = 0.$$

Also folgt aus (I)

$$\lambda + \sigma + 1 = 0$$

$$\text{d. h.}^{(1)} \quad \left\{ \begin{array}{c} 12 \\ 1 \end{array} \right\} + \left\{ \begin{array}{c} 12 \\ 2 \end{array} \right\} = 1.$$

(4)

Der Inhalt S des Flächenstückes ist durch

$$S = \iint \sqrt{F \bar{G} - F^*} \, du \, dv$$

dargestellt, wobei E, F, G die ersten Fundamentalgrößen sind. Somit ergibt sich aus (I) in §1:

$$\begin{aligned} S &= \frac{1}{2} \iint \lambda (E_v F - G_u E) \, du \, dv \\ &= \frac{1}{2} \iint \frac{\lambda}{\sigma} (F G_u - G E_v) \, du \, dv. \end{aligned}$$

(1) BLASCHKE, W.: Vorlesungen über Differential-geometrie, I, Berlin (1930) S. 115.

Wenn λ , σ konstant ist, dann ergibt sich

$$\begin{aligned} S &= \frac{\lambda}{2} \iint (E_v F - G_u E) du dv \\ &= \frac{\lambda}{2\sigma} \iint (F G_u - G E_v) du dv. \end{aligned}$$

(5)

Betrachten wir LIOUVILLSche Fläche,⁽¹⁾ dann folgt:

$$(1) \quad E=U+V, \quad G=U+V, \quad F=0,$$

also

$$(2) \quad \sigma = \frac{V'}{U}, \quad -\frac{1}{2} \lambda = -\frac{U+V}{U'}.$$

Setzen wir (2) in (I) in § 1 ein, dann folgt:

$$D(x) = x_u - \frac{V'}{2(U+V)} x_v - \frac{U'}{2(U+V)} x_t = 0.$$

Also werden h und k geschrieben in der Form:⁽²⁾

$$h = \frac{3 U' V'}{4 (U+V)^2}, \quad k = \frac{3 U' V'}{4 (U+V)^2}.$$

Also folgt der

Satz: In LIOUVILLEScher Fläche sind zwei Invarianten h und k einander gleich.

(6)

Es werde ein dem Polarkoordinatensystem der Ebene analoges System eingeführt.

Der Pol o liege auf der, Kugeloberfläche, u sei der sphärische

(1) EISENHART, L. P.: A treatise on the Differential-geometry of Curves and Surfaces, Princeton (1909) p. 214.

(2) l. c. (1) p. 406.

Abstand des betreffenden Punktes von o , v der Winkel, den u mit einem Anfangsmeridian bildet, beide im Bogenmass gemessen.

u geht also von o bis π , v dagegen kann alle Werte annehmen, auch grösser werden als 2π .

Der Kugelradius sei gleich Eins. Dann lauten die Gleichungen der Kugel:

$$(1) \quad \begin{cases} \xi = \sin u \sin v, \\ \eta = \sin u \cos v, \\ \varphi = \cos u. \end{cases}$$

Die Gauss'schen Fundamentalgrössen sind:

$$(2) \quad \begin{cases} E = \sum \left(\frac{\partial \xi}{\partial u} \right)^2 = 1, \\ F = \sum \frac{d\xi}{du} \frac{d\xi}{dv} = 0, \\ G = \sum \left(\frac{d\xi}{dv} \right)^2 = \sin^2 u. \end{cases}$$

Das Linienelement einer beliebigen Kurve auf der Fläche lautet dann:

$$ds^2 = du^2 + \sin^2 u \cdot dv^2.$$

Setzen wir (2) in (I) in § 1 ein, dann folgt

$$(3) \quad \tan u \cdot \eta_u = \eta + f(u),$$

weil

$$\sigma = 0, \lambda = -\tan u$$

ist. Aus (3) kann man die Invarianten h, k leicht berechnen. Betrachten wir die Fläche, deren Linienelement ist

$$ds^2 = du^2 + e^{2u} dv^2,$$

dann ist

$$\sigma = 0, \lambda = -1,$$

also erfolgt aus (I) in § 1

$$\xi_{uv} = \xi_v,$$

oder

$$\xi_u = \xi + f(u),$$

d.h. (3)

$$\xi = \varphi(v) e'' + \Phi(u),$$

wobei φ , Φ beliebige Funktionen sind.⁽¹⁾

(3) Eiflächenpaare.

Wir wollen den folgenden Satz beweisen.

Satz: *Haben zwei Eiflächen in allen Richtungen verschiedene Breite nur in konstanten Verhältnissen, so haben sie in allen Richtungen auch verschiedene Umfänge nur in konstanten Verhältnissen und umgekehrt.*

Beweis: ϕ , δ seien Polarkoordinaten auf dem sphärischen Bild. $p(\delta, \phi)$ und $q(\delta, \phi)$ seien die Abstände vom Ursprung bis zu den Tangentenebenen an den beiden Eiflächen; es ist dann

$$(1) \quad \begin{cases} p(\delta, \phi) = \sum_0^\infty r X_r(\delta, \phi), \\ q(\delta, \phi) = \sum_0^\infty r Y_r(\delta, \phi). \end{cases}$$

Hieraus entwickelt sich nach Kugelflächenfunktionen:

(1) NAKAJIMA, S.: Ueber zwei Flächen, welche eine Beziehung haben I), II), (III), (IV), (V), (VI), Tôhoku Math. Journ. Vol. 30, p. 142; Vol. 33, p. 153, p. 157; Vol. 35, p. 329; Vol. 36, p. 125, p. 257.

MATSUMURA, S.: On some differential Equations Journ. of the Society of tropical Agriculture, IV, V: Vol. V, p. 62, p. 229, u. s. w.

Tôkyô Butsurigakko-Yassai 461 p. 169; 467 p. 416; 488 p. 319, u. s. w.

MATSUMURA, S.: Über Flächen und Kurven (II), Mem. of the Fac. of Sci. and Agr., Teihoku Imp. Univ., Vol. V (1933), p. 219.

MATSUMURA, S.: Über Flächen und Kurven (III), Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ., Vol. V (1933), p. 290.

$$(2) \quad \begin{cases} p(\delta, \phi) + p(\pi - \delta, \pi + \phi) = 2 \sum_0^{\infty} X_{2\lambda}(\delta, \phi), \\ q(\delta, \phi) + q(\pi - \delta, \pi + \phi) = 2 \sum_0^{\infty} Y_{2\lambda}(\delta, \phi). \end{cases}$$

Sollen beide Flächen in jeder Richtung verschiedene Breiten nur in konstanten Verhältnissen besitzen, so muss

$$(3) \quad X_{2\lambda} = k Y_{2\lambda} (\lambda = 0, 1, 2, \dots)$$

sein,⁽¹⁾ wobei k eine Konstante ist.

$U(\delta, \phi)$ und $V(\delta, \phi)$ seien die Umfänge der beiden Eiflächen in der Richtung (δ, ϕ) d.h. die Umfänge der senkrechten ebenen Projektionen in der Richtung des Kugelradius zum Punkte (δ, ϕ) auf dem sphärischen Bilde.

Bezeichnet man mit P_n das n -te Legendresche Polynom, so erhält man nach Minkowski zunächst

$$\begin{aligned} U(0, \phi) &= \int_0^{2\pi} p\left(\frac{\pi}{2}, \phi\right) d\phi = \sum_{\tau=0}^{\infty} X_{2\tau}\left(\frac{\pi}{2}, \phi\right) d\phi \\ &= 2\pi \sum_{\lambda=0}^{\infty} P_{2\lambda}(0) Y_{2\lambda}(0, \phi) \end{aligned}$$

und im allgemeinen

$$(4) \quad \begin{cases} U(\delta, \phi) = 2\pi \sum_{\lambda=0}^{\infty} P_{2\lambda}(0) X_{2\lambda}(\delta, \phi), \\ V(\delta, \phi) = 2\pi \sum_{\lambda=0}^{\infty} P_{2\lambda}(0) Y_{2\lambda}(\delta, \phi). \end{cases}$$

Aus (3) folgt aber nach (4) zunächst unsere erste Behauptung:

$$(5) \quad U(\delta, \phi) = k V(\delta, \phi).$$

Umgekehrt folgt aus (4) und (5) aber wieder (3) und somit wegen (2) auch die Umkehrung der ersten Behauptung, w.z.b.w.

N. B. Wenn eine geschlossene Fläche durch eine durch einen festen Punkt hindurchgehende Ebene in eine Ebenekurve geschnitten

(1) NAKAJIMA, S.: Eiflächenpaare gleicher Breiten und gleicher Umfänge, Japanese Journ. of Math., Vol. VII (1930) p. 225.

wird, so ist der Inhalt der Schnittkurve durch die zur Richtung (∂, ϕ) senkrechte Ebene

$$2\pi \sum_{\lambda=0}^{\infty} P_{2\lambda}(0) Y_{2\lambda}(\partial, \phi)$$

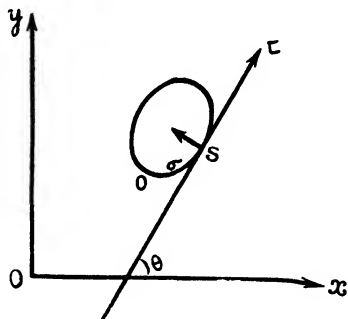
gleich.⁽¹⁾

Also ist der obere Satz auch auf Breite und Inhalt von Schnittebenekurve der Eifläche anwendbar oder auf das Ebenenkurvenpaar (Eilinen).

(4) Ueber die Deviation einer Ebenenkurve

Wir werden im allgemeinen einen variablen Punkt der gegebenen Kurve mit s und seine Bogenlänge mit σ bezeichnen, und zwar in solcher Weise, dass σ von einer festen Marke o aus gerechnet wird in der Richtung der Tangente (vgl. d. Figur).

Sind σ und $\sigma + d\sigma$ die Bogenlängen zwei aneinanderfolgender Punkte, ferner θ und $\theta + d\theta$ die Azimuthe der diesen Punkten entsprechenden Tangenten, so gilt bekanntlich für den Krümmungsradius ρ , den die Kurve an dieser Stelle besitzt, die Formel:



$$(1) \quad \frac{1}{\rho} = \varepsilon \frac{d\theta}{d\sigma},$$

wo $\varepsilon = +1$ oder -1 ist, je nachdem der Krümmungsmittelpunkt auf der innern oder äussern Normale sitzt.

Schon habe ich

$$(2) \quad \tan \varphi = -\frac{1}{3} \frac{d\rho}{d\sigma}$$

(1) KUBOTA, T.: Einige Probleme über knovex-geschlossene Kurven und Flächen
Tôhoku Math. Journ. Vol. 17 (1920) p. 360.

bewiesen.⁽¹⁾

Aus (1), (2) folgt⁽²⁾

$$(3) \quad \varepsilon \tan \varphi = \frac{1}{3\rho} \frac{d\rho}{d\theta}.$$

Aus (3) kann man wissen, dass, wenn ρ unabhängig von θ ist, dann *der Krümmungsmittelpunkt von s der Affinkrümmungsmittelpunkt und der Punkt s auf einer Geraden liegen.*

Im folgenden betrachten wir kaustische Kurve (c) und setzen

$$\delta = Ks.$$

wobei K den *Berührungspunkt in s mit (c)* bezeichnet, dann folgt

$$(4) \quad \delta = \frac{\rho \cos \theta}{2} = \frac{1}{2} \frac{d\sigma}{d\theta} \cos \theta = \frac{1}{2} \frac{dx}{d\theta},$$

wobei (x, y) die Kartesischen Koordinaten von dem Punkt s sind.

Aus (1), (4) folgt

$$\delta = \frac{1}{2} \rho \frac{dx}{d\sigma}.$$

Nehmen wir⁽²⁾

$$(5) \quad \tan \varphi = \frac{1}{3} \cdot \frac{d\rho}{ds} - \frac{\rho}{c},$$

dann folgt aus (1)

$$(6) \quad \frac{1}{\Delta(s)} = \varepsilon \frac{d\theta}{d\sigma},$$

weil sich aus (5) ergibt:

$$(7) \quad \rho \equiv \Delta(s) = 3 \exp. (3s/c) \cdot \int \tan \varphi \cdot \exp. (3s/c) ds.$$

(1) MATSUMURA, S.: Ueber einen affingeometrischen Satz und die Deviation ebener Kurven, Tôhoku Math. Journ. vol. 36 (1933) p. 189.

(2) ROTHE, R.: Aufgabe aus der Kurventheorie, Archiv der Mathematik und Physik III Reihe XXVIII, Heft 3/4, S. 171.

Man kann die beiden Arbeiten von Kubota⁽¹⁾ und Takasu⁽²⁾ durch (5) etwas modifizieren.⁽³⁾

Es sei $p(\theta)$ die Stützgeradenfunktion der C'' Klasse von einer konvex-geschlossenen Kurve C in R_2 , dann ist die Bedingung dafür, dass C eine Mittelpunktkurve ist, mit

$$(8) \quad \nabla(\theta) = \nabla(\theta + \pi)$$

bezeichnet, wobei

$$(9) \quad \begin{aligned} \rho &= \Delta(s) = \nabla(\theta), \\ p(\theta) + p''(\theta) &= \nabla(\theta) \end{aligned}$$

ist und zwar θ den Winkel zwischen der Normale zur Stützgerade und der x Achse bedeutet.

Wenn

$$(10) \quad \int_{\theta}^{\theta + \pi - \alpha} \nabla dt = \text{const.},$$

für alle θ besteht, dann muß C ein Kreis oder eine periodische Kurve sein,⁽⁴⁾ wo $\pi = 3.1459 \dots$ und α eine Konstant ist.

Wenn C eine Konstantbreitkurve ist, dann ergibt sich

$$(11) \quad \nabla(\theta) + \nabla(\theta + \pi) = \text{const.}$$

Einen Kegelschnitt nennt man jede Kurve, die im gewöhnlichen Kartesischen System mit unbeweglichen Achsen durch eine Gleichung zweiten Grades zwischen den Koordinaten x und y ihrer Punkte dargestellt wird.

Wird der Kegelschnitt auf die Tangente und die Normale in einem beliebigen Punkte s bezogen, so fehlt in der Gleichung das absolute Glied.

- (1) KUBOTA, T.: Beiträge zur Inversionsgeometrie und Laguerre-Geometrie, Japanese Journal of Mathematics, Vol. I. (1924) p. 41.
- (2) TAKASU, T.: Natural Equations of Curves under circular point Transformation Group and their Dauls, I, Japanese Journ. of Math. Vol. I. (1924) p. 52.
- (3) MATSUMURA, S.: Beiträge zur Inversionsgeometrie und Laguerre-Geometrie, Tôhoku Math. Journ. Vol. 37 (1933) p. 468.
- (4) NAKAJIMA, S.: On some characteristic Properties of curves and Surfaces, Tohoku Math. Journ. 18 (1920), p. 272.

Aus

$$(12) \quad p''(\theta) + p(\theta) = \nabla(\theta)$$

folgt

$$(13) \quad p(\theta) = \sin \theta \left[\int_0^\theta \nabla(t) \cos t \, dt + C_1 \right] - \\ - \cos \theta \left[\int_0^\theta \nabla(t) \sin t \, dt + C_2 \right],$$

wobei C_1, C_2 zwei Konstanten sind.

So ist

$$(14) \quad q^2(\varphi) + p^2(\theta) q(\varphi) \sin(\theta - \varphi) = \\ p^2(\theta) + p(\theta) q'(\varphi) \sin(\varphi - \theta)$$

die *Bedingung dafür, dass zwei Eilini*⁽¹⁾

$$p(\theta) \text{ und } q(\varphi)$$

ein rhombisches Netz bilden, wobei (13) und

$$(15) \quad q(\varphi) = \sin \varphi \left[\int_0^\varphi \nabla(t) \cos t \, dt + C_1 \right] - \\ - \cos \varphi \left[\int_0^\varphi \nabla(t) \sin t \, dt + C_2 \right]$$

bestehen.⁽²⁾

(1) PERRON, O.: Bestimmung aller geradlinigen rhombischen Netze, Sitzungsberichte der Bayerischen Akademie der Wissenschaften, München (1925).

(2) NAKAJIMA, S.: On Ovals, Journ. of the Math. Association of Japan for secondary Education, Vol. 13, p. I.

Beiträge zur Geometrie der Kreise und Kugeln (VIII)

Sôji MATSUMURA

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(1)

Wir können die Kreisscharen im Lobatschewskyschen Raum auf dieselbe Weise wie in meiner Arbeit begründen.

Hier gehen wir von vier reellen Zahlen

$$x_0, x_1, x_2, x_3, x_4$$

aus, zwischen denen die Beziehung besteht :

$$(1) \quad (x|x) = x_0 - x_1 - x_2^2 - x_3^2 - x_4^2 = 0.$$

In unserem Raume ist eine Ebene gegeben durch eine lineare Gleichung :

$$(\hat{z}|x) = \hat{z}_0 x_0 - \hat{z}_1 x_1 - \hat{z}_2 x_2 - \hat{z}_3 x_3 - \hat{z}_4 x_4 = 0.$$

Jetzt brauchen wir in THOMSENS¹⁾ Entwicklung nur x_1, x_2, x_3 der Reihe nach durch $x_1 i, x_2 i, x_3 i$ zu ersetzen. Sind die Gleichungen zweier Ebenen

$$\sum_{i=0}^4 A_i x_i = 0 \text{ und } \sum_{i=0}^4 B_i x_i = 0$$

in der Normalform gegeben, so stellt der Ausdruck

(1) THOMSEN, G.: Ueber Kreisflächen und Kurven in der Ebene und ueber Kugelscharen und Kurven im Raum, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) p. 137.

[Mem. of the Fac. of Sci. and Agr., Taihoku Imp Univ., Formosa, Japan, Vol. X, No. 2, November 1933.]

$$(2) \quad A_0 B_0 - A_1 B_1 - A_2 B_2 - A_3 B_3 - A_4 B_4$$

eine Invariante der beiden Ebenen dar, die geeignet ist, die gegenseitige Lage der beiden Ebenen zu charakterisieren.

Die Ebenen können nur dann Punkte gemeinsam haben, wenn der Ausdruck (z) seinem absoluten Betrage nach kleiner ist als eins.

Speziell sagen wir, die Ebenen stehen zueinander senkrecht, wenn der Ausdruck (z) den Wert Null hat.

Wir gehen von zwei zueinander senkrecht stehenden Ebenen A und B aus, deren Gleichungen in der Normalform gegeben sind; es soll also sein:

$$A_0^2 - A_1^2 - A_2^2 - A_3^2 - A_4^2 = 0,$$

$$B_0^2 - B_1^2 - B_2^2 - B_3^2 - B_4^2 = 0,$$

$$A_0 B_0 - A_1 B_1 - A_2 B_2 - A_3 B_3 - A_4 B_4 = 0.$$

Dann kann jede durch die Schnittlinie gehende Ebene durch folgende Gleichung dargestellt werden

$$(\rho A_0 + \sigma B_0) x_0 + (\rho A_1 + \sigma B_1) x_1 + (\rho A_2 + \sigma B_2) x_2 + \\ + (\rho A_3 + \sigma B_3) x_3 + (\rho A_4 + \sigma B_4) x_4 = 0,$$

und diese erscheint in der Normalform, wobei ρ, σ zwei Parameter sind.

Aus (z) für zwei Ebenen ergeben sich drei Möglichkeiten:

a) für

$$-1 < A_0 B_0 - A_1 B_1 - \dots - A_4 B_4 < 1$$

haben die Ebenen eine Gerade gemeinsam

b) für

$$(A_0 B_0 - A_1 B_1 - A_2 B_2 - A_3 B_3 - A_4 B_4)^2 = 1$$

haben sie sich nicht schneidende Ebenen.

c) für

$$(A_0 B_0 - A_1 B_1 - A_2 B_2 - A_3 B_3 - A_4 B_4) = \pm 1$$

parallele Ebenen.

(2)

(1) Betrachten wir die zwei Kreisflächen⁽¹⁾

$$(1) \quad \mathfrak{x} = \phi(t, \tau), \quad \bar{\mathfrak{x}} = \psi(t, \tau);$$

die Bedingung dafür, dass zwei Systeme von Kurven

$$\phi = \text{const.},$$

und

$$\psi = \text{const.}$$

senkrecht sind, ist die, dass⁽²⁾

$$\nabla \phi \cdot \nabla \psi = 0.$$

ist.

Der Vektor

$$(2) \quad \nabla = ((\theta_\tau, \theta_\tau) \phi_1 - (\theta_t, \theta_\tau) \phi_2) r_1 + ((\theta_t, \theta_t) \phi_2 - (\theta_t, \theta_\tau) \phi_1) r_2$$

ist parallel zu $\nabla \phi$.

Die Bedingung dafür, dass zwei Flächen (1) applikabel sind;⁽³⁾

$$(\nabla \phi)^2 = (\nabla' \phi')^2, \quad (\nabla \psi)^2 = (\nabla' \psi')^2,$$

ist die, dass

$$\nabla \phi \cdot \nabla \psi = \nabla' \phi' \cdot \nabla' \psi'$$

ist.

(2) Es seien vier Kreise $\mathfrak{R}_1, \mathfrak{R}_2; \bar{\mathfrak{R}}_1, \bar{\mathfrak{R}}_2$ im R_3 gegeben.

$\mathfrak{y} = \rho_\alpha(t) \mathfrak{x}^\alpha(t)$ sei eine normierte Kugel durch \mathfrak{R}_1 , dann folgt

$$\cos^2 \varphi = T^{\alpha\beta}(t) \rho_\alpha(t) \rho_\beta(t),$$

$$(1) \quad (\mathfrak{y} \mathfrak{y}) = \rho_\alpha \rho_\beta (\mathfrak{x}^\alpha \mathfrak{x}^\beta) = 1,$$

wobei φ den Winkel zwischen \mathfrak{y} und \mathfrak{R}_2 bedeutet.

(1) MATSUMURA, S.: Beiträge zur Geo. der Kreise und Kugeln (I), Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ., Formosa, Japan, Japan, Vol. V, 74.

(2) WEATHERBURN, C. E.: Differentialgeometry of three Dimensions (I), Cambridge (1928) p. 222.

(3) l. c. (2) (II) p. 189.

Wenn $\cos^2 \varphi = k^2$ ist, so folgt

$$(2) \quad (T^{\alpha\beta}(t) - k^2 A^{\alpha\beta}(t)) \rho_\alpha(t) \rho_\beta(t) = 0.$$

Es sei

$$\bar{\eta} = \bar{\rho}_\lambda(t) \bar{\xi}^\lambda(t)$$

eine normierte Kugel durch $\bar{\mathfrak{R}}_1$, dann folgt

$$(3) \quad \begin{aligned} \cos^2 \bar{\varphi} &= \bar{T}^{\alpha\beta}(t) \bar{\rho}_\alpha(t) \bar{\rho}_\beta(t), \\ (\bar{\eta} \bar{\eta}) &= \bar{A}^{\lambda\mu} \bar{\rho}_\lambda(t) \bar{\rho}_\mu(t) = 1, \end{aligned}$$

wobei $\bar{\varphi}$ den Winkel zwischen $\bar{\eta}$ und $\bar{\mathfrak{R}}_2$ bedeutet.

Wenn $\cos^2 \bar{\varphi} = \bar{k}^2$ ist, so folgt

$$(4) \quad (\bar{T}^{\lambda\mu}(t) - \bar{k}^2 \bar{A}^{\lambda\mu}(t)) \bar{\rho}_\lambda(t) \bar{\rho}_\mu(t) = 0.$$

Ist η senkrecht zu der Kugel $\bar{\eta}$ und zu der benachbarten Kugel

$$\bar{\eta} + \frac{d\bar{\eta}}{dt} dt,$$

dann folgen

$$(5) \quad \rho_\alpha \bar{\rho}_\lambda (\xi^\alpha \bar{\xi}^\lambda) = 0,$$

$$(6) \quad \rho_\alpha \dot{\rho} (\xi^\alpha \bar{\xi}^\lambda) + \rho_\alpha \bar{\rho}_\lambda (\xi^\alpha \dot{\bar{\xi}}^\lambda) = 0,$$

wo • bezeichnet, durch t zu differenzieren.

Damit solche Kugeln η und $\bar{\eta}$ existieren, müssen $\rho_\alpha, \bar{\rho}_\lambda$ (1), (2), (3), (4), (5), (6) erfüllen.

(3)

(1) Wollte man nachweisen, dass zwei Flächen

$$(1) \quad \xi = \overset{0}{\xi}(t, \tau), \quad \bar{\xi} = \overset{1}{\xi}(t, \tau),$$

die durch gleiche Parameterwerte einander längentreu abgebildet sind, durch eine stetige Verbiegung ineinander übergeführt werden können, so müsste man sich eine einparametrische Flächenschar

$$(2) \quad \bar{x} = x(t, \tau, \lambda)$$

darartig verschaffen, dass etwa zum Wert $\lambda=0$ des Scharparameters die Fläche \bar{x}_0 und zum Wert $\lambda=1$ die Fläche \bar{x}_1 gehört.

Man wird dabei natürlich verlangen, dass alle Flächen der Schar (2) den beiden Ausgangsflächen (1) längentreu abgebildet sind.

Wir beschränken uns auf die Untersuchung der bereits sehr aufschlussreichen infinitesimalen Verbiegungen der Ausgangsfläche $x = \bar{x}_0$ die wir uns als Fläche $t=0$ in der Schar (2) eingebettet denken.

Dabei seien die Funktionen $x(t, \tau, \lambda)$ in einer Umgebung des Streifens $\lambda=0$ mindestens dreimal nach den t oder τ und mindestens einmal nach λ stetig differentizierbar.

Dann ist

$$(3) \quad x(t, \tau, \lambda) = x(t, \tau, 0) + \lambda \frac{\partial}{\partial \lambda} x(t, \tau, 0)$$

eine Näherungsdarstellung der Schar für kleine λ , also eine Darstellung der infinitesimalen Verbiegung der Ausgangsfläche.

Wir setzen zur Abkürzung

$$(4) \quad \bar{x}(t, \tau, \lambda) = \bar{x}, \quad x(t, \tau, 0) = x, \quad \frac{\partial}{\partial \lambda} x(t, \tau, 0) = z;$$

(3) lautet dann

$$(5) \quad \bar{x} = x + \lambda z.$$

Daraus folgt durch Differentiation für entsprechende Richtungen auf \bar{x} und x

$$(6) \quad d\bar{x} = dx + \lambda dz.$$

Sollen die beiden Flächen \bar{x} und x einander längentreu abgebildet sein, so müssen die quadrierten Bogenelemente $d\bar{s}^2$ und ds^2 von \bar{x} und x identisch in den t oder τ und dt oder $d\tau$, d.h. für alle entsprechenden Richtungen in allen entsprechenden Punkten übereinstimmen.

Nun ist

$$(7) \quad d\bar{s}^2 = d\bar{x} d\bar{x} = dx dx + 2\lambda dx dz = ds^2 + 2\lambda dx dz,$$

wobei wir Glieder mit λ^2 vernachlässigen.

Also muß

$$(8) \quad d\mathfrak{x} \cdot d\mathfrak{z} = 0$$

sein. Diese Relation besitzt eine einfache geometrische Deutung, wenn wir den Vektor \mathfrak{z} als Ortsvektor einer zweiten Fläche deuten; sie besagt dann, dass entsprechende Linienelemente auf den beiden Flächen \mathfrak{x} und \mathfrak{z} stets zueinander senkrecht stehen.

Man sagt dann, *die beiden Flächen entsprechen einander durch Orthogonalität der Elemente*.

(2) Es sei eine Kugelkongruenz

$$\varphi = \varphi(u^1, u^2), (\varphi \varphi) = 1$$

und die beiden Envelopenmäntel davon

$$\mathfrak{x} = \mathfrak{x}(u^1, u^2), ((\mathfrak{x} \mathfrak{x}) = 0)$$

$$\bar{\mathfrak{x}}, ((\bar{\mathfrak{x}} \bar{\mathfrak{x}}) = 0)$$

vorgegeben, wobei $\bar{\mathfrak{x}}$ ein fester Punkt ist.

Man führe die Bezeichnung

$$\frac{\partial \varphi}{\partial u^i} = \varphi_i$$

ein.

Die betreffenden Koordinaten seien wie folgt normiert:

$$(\mathfrak{x} \bar{\mathfrak{x}}) = 2k^2.$$

Man lege die folgenden Bezeichnungen und Formeln zu Grunde:

$$(\varphi_h \varphi_k) du^h du^k = g_{hk} du^h du^k$$

(Grundform der Tensorrechnung),

$$g = g_{11} g_{22} - g_{12}^2, D_{hk} = -(\varphi_h \mathfrak{x}_k) = (\varphi \mathfrak{x}_{hk}) = (\mathfrak{x} \varphi_{hk}),$$

$$D_{hk} = 0$$

$$E^{11} = 0, E^{12} = g^{-\frac{1}{2}} = -E^{21}, E^{22} = 0,$$

$$g^{hk} = \frac{\partial}{\partial g_{hk}} - \log g, N_h = -(\bar{\mathfrak{x}} \mathfrak{x}_h) = 0$$

$$D = D_{11} D_{22} - D_{12}^2, \bar{D} = \bar{D}_{11} \bar{D}_{22} - \bar{D}_{12}^2,$$

weil, wenn $\bar{x} = \bar{x}(u, v)$ ist, dann folgt

$$(\bar{x} \varphi) = 0,$$

d.h.

$$(\bar{x}_{hk} \varphi) + (\bar{x}_k \varphi_h) + (\bar{x}_h \varphi_k) + (\bar{x} \varphi_{hk}) = 0,$$

$$\begin{aligned} \therefore \quad \bar{D}_{hk} &= -(\varphi_h \bar{x}_k) = (\varphi \bar{x}_{hk}) = (\bar{x} \varphi_{hk}) \\ &= -(\varphi \bar{x}_k) = (\varphi \bar{x}_{hk}) = -(\bar{x}_h \varphi_k), \end{aligned}$$

wobei \mathfrak{R}^g die Gaussche Krümmung der Grundform $g_{hk} du^h du^k$ ist.

Dann entstehen die folgenden Ableitungsgleichungen

$$\varphi_{hk} = -g_{hk} \varphi + D_{hk} \frac{\bar{x}}{2k^2},$$

$$\bar{x}_h = -D_h^* \varphi.$$

Die Integrabilitätsbedingungen dieser Differentialgleichungen sind:

$$\mathfrak{R}^g = 1$$

d.h. \mathfrak{R}^g ist konstant.

Führt man nun die krümmungslinigen Parameter ein, so ist es für die Ribaucourschen Kugelskongruenzen kennzeichnend, dass

$$\bar{g}_{12} = D_{12} = 0$$

gilt.⁽¹⁾

(4)

(1) Es sei K eine durch die Gleichungen

$$\bar{x}^a(t) = x^a(t) + \varepsilon \omega^a(t), \quad (\bar{x}^a \bar{x}^a) = 0, \quad (x^a x^a) = 0,$$

$$(\omega^a \omega^a) = 0, \quad (a = \text{I, II, III}),$$

definierte reelle Kurve, wo t ein reeller Parameter ist.

Es seien P_0 und P_1 die den Parameterwerten t_0 bzw. t_1 entsprechenden Punkte auf K.

(1) Vergl. TAKASU, T.: Differential Kugelgeometrie, III, The Science Reports of the Tôhoku Imp. Univ. Vol. XXI, (1932) p. 603.

Wenn ω^α eine Funktion von t in der Art ist, dass für $t=t_0, t_1$,

$$(1) \quad \omega^\alpha = 0$$

ist, dann definieren die Gleichungen

$$\bar{x}^\alpha = x^\alpha + \varepsilon \omega^\alpha$$

eine durch P_0 und P_1 hindurchgehende Nachbarkurve \bar{k} von k , wenn ε eine Infinitesimale ist.

Wir betrachten

$$(2) \quad I = \int_{t_0}^{t_1} \varphi(x^I, x^{II}, x^{III}, \dot{x}^I, \dot{x}^{II}, \dot{x}^{III}) dt, \quad \left(\dot{x} = \frac{dx}{dt} \right),$$

wo φ eine analytische Funktion von x^I, x^{II} und x^{III} ist.

Es sei \bar{I} der \bar{k} entsprechende Wert von I .

Dann gilt wegen des Taylorschen Satzes:

$$\bar{I} - I = \varepsilon \int_{t_0}^{t_1} \left[\frac{\partial \varphi}{\partial x^\alpha} \omega^\alpha + \frac{\partial \varphi}{\partial \dot{x}^\alpha} \dot{\omega}^\alpha \right] dt + \dots, \dots,$$

wo

$$\dot{\omega}^\alpha = \frac{\partial \omega^\alpha}{\partial x^\beta} \dot{x}^\beta$$

ist, und der punktligne Teil aus Infinitesimalen höherer Ordnung in ε besteht.

Schreibt man

$$(3) \quad \delta I = \varepsilon \int_{t_0}^{t_1} \left[\frac{\partial \varphi}{\partial x^\alpha} \omega^\alpha + \frac{\partial \varphi}{\partial \dot{x}^\alpha} \dot{\omega}^\alpha \right] dt$$

und integriert das zweite Glied nach Teilen, so erhalten wir nach (1)

$$(4) \quad \delta I = \varepsilon \int_{t_0}^{t_1} \left[\frac{\partial \varphi}{\partial x^\alpha} - \frac{d}{dt} \left(\frac{\partial \varphi}{\partial \dot{x}^\alpha} \right) \right] \omega^\alpha dt.$$

Die notwendige und hinreichende Bedingung dafür, dass K die Extremale ist, besteht darin, dass

$$(5) \quad -\frac{d}{dt} \left(-\frac{\partial \varphi}{\partial \dot{x}^\alpha} \right) - \frac{\partial \varphi}{\partial x^\alpha} = 0$$

bestehen, die die sogenannten Eulerschen Gleichungen sind.

Zu weiteren Untersuchungen vergleiche man mit EISENHARTS Buch.⁽¹⁾

(2) Es seien ξ, η zwei Kreise im R_2 , dann wird mit

$$(1) \quad \bar{\xi} = \xi - (\xi \eta) \eta, \quad (\eta \eta) = 1$$

ein Kreis in R_2 durch die Schnittpunkte von ξ und η bezeichnet.

Aus (1) folgt

$$(2) \quad (\bar{\xi} \eta) = (\xi \eta) - (\xi \eta) = 0,$$

so folgt der

Satz: Ein Kreis $\bar{\xi}$ ist senkrecht zu dem Kreis η .

$$(3) \quad \bar{\eta} = \eta - (\eta \eta) \eta, \quad (\eta \eta) = 1$$

sind ein Kreis in R_2 durch die Schnittpunkte von zwei Kreisen η und η und senkrecht zu dem Kreis η ; so folgt der

Satz: Zwei Kreise $\bar{\xi}, \bar{\eta}$ sind senkrecht zu η .

Aus (1), (3) ergibt sich

$$\begin{aligned} (\bar{\xi} \bar{\eta}) &= (\xi - (\xi \eta) \eta, \eta - (\eta \eta) \eta) \\ &= (\xi \eta) - (\xi \eta) (\eta \eta) - (\eta \eta) (\xi \eta) + (\xi \eta) (\eta \eta) \\ &= (\xi \eta) - (\eta \eta) (\xi \eta). \end{aligned}$$

So folgt der

Satz: Die Bedingung dafür, dass $\bar{\xi}$ und $\bar{\eta}$ zueinander senkrecht sind, ist die

$$(4) \quad (\xi \eta) = (\eta \eta) (\xi \eta).$$

So folgt der

Satz: (4) ist die Bedingung dafür, dass drei Kreise $\bar{\xi}, \bar{\eta}, \eta$ zueinander senkrecht sind.

Nehmen wir

(1) EISENHART, L. P.: RIEMANNian Geometry, Princeton (1926).

$$(5) \quad \bar{\bar{x}} = x - (x \eta) \eta, \quad (\eta \eta) = 0,$$

so folgt

$$(6) \quad (\bar{\bar{x}} \eta) = (x \eta),$$

d.h. η bildet zu $\bar{\bar{x}}$ und x einen gleichen Winkel.

Betrachten wir

$$(7) \quad \bar{\bar{y}} = y - (y \eta) \eta, \quad (\eta \eta) = 0,$$

so folgt

$$(\bar{\bar{x}} \bar{\bar{y}}) = (x y) - 2 (x \eta) (y \eta).$$

Also folgt der

Satz: $(x y) = 2 (x \eta) (y \eta)$ ist die Bedingung dafür, dass zwei Kreise $\bar{\bar{x}}$ und $\bar{\bar{y}}$ zueinander senkrecht sind.

Dehnen wir unsere LIES Geometrie auf

$$\langle \bar{\bar{x}}^I \bar{\bar{x}}^{II} \rangle = 0$$

aus, so folgt

$$(\bar{\bar{x}}^I - \bar{\bar{x}}^{II})^2 + (\eta^I - \eta^{II})^2 + (\varphi^I - \varphi^{II})^2 = -4,$$

wo

$$x_0 = \frac{1 + \xi^2 + \eta^2 + \varphi^2}{2}, \quad x_1 = \frac{1 - (\xi^2 + \eta^2 + \varphi^2)}{2}, \quad x_2 = \xi,$$

$$x_3 = \eta, \quad x_4 = \varphi, \quad x_5 = 1.$$

(5)

Es seien zwei Kreise \mathfrak{R} , \mathfrak{S} im R_s gegeben.

Ist $y = \rho_\alpha \bar{x}^\alpha$ eine normierte Kugel im R_s durch \mathfrak{R} , so setzen wir

$$(1) \quad y y = \rho_\alpha \rho_\beta A^{\alpha\beta} = 1.$$

Dann muss sein

$$(2) \quad \cos^2 \varphi = T^{\alpha\beta} \rho_\alpha \rho_\beta,$$

wobei φ den Winkel zwischen y und \mathfrak{S} bedeutet.

Wenn $\bar{\mathfrak{R}}$ immer auf \mathfrak{y} liegt, d.h. $\varphi \equiv 0$ ist, so folgt aus (2)

$$T^{\alpha\beta} \rho_\alpha \rho_\beta \equiv 1,$$

d.h. es muß sein

$$(3) \quad A^{\alpha\beta} \equiv T^{\alpha\beta}$$

Betrachten wir eine andere Kugel $\bar{\mathfrak{y}} = \rho_\beta \xi^\beta$ statt $\mathfrak{y} = \rho_\alpha \xi^\alpha$ durch \mathfrak{R} , dann folgt statt (2)

$$(4) \quad \cos^2 \bar{\varphi} = T^{\alpha\beta} \rho_\alpha \rho_\beta$$

wobei $\bar{\varphi}$ den Winkel zwischen $\bar{\mathfrak{y}}$ und $\bar{\mathfrak{R}}$ bedeutet.

Um zu finden den Wert von

$$(5) \quad \cos(\mathfrak{y} \bar{\mathfrak{y}}) \equiv \rho_\alpha \rho_\beta \xi^\alpha \xi^\beta,$$

muss man aus (2), (4) und (5) ρ vertreiben.

Es seien die Kreise $\mathfrak{R}, \bar{\mathfrak{R}}, \bar{\bar{\mathfrak{R}}}, \dots$ im R_3 gegeben.

Ist $\mathfrak{y} = \rho_\alpha \xi^\alpha$ eine normierte Kugel im R_3 durch \mathfrak{R} , dann muss sein

$$(6) \quad \begin{cases} \cos^2 \varphi = T^{\alpha\beta} \rho_\alpha \rho_\beta, \\ \cos^2 \varphi_1 = T^{\alpha\beta} \rho_\alpha \rho_\beta, \\ \dots\dots\dots \end{cases}$$

wobei

$$\varphi_1 \equiv (\mathfrak{y} \bar{\mathfrak{R}}), \varphi_2 \equiv (\mathfrak{y} \bar{\bar{\mathfrak{R}}}), \dots\dots\dots$$

ist.

Wenn

$$\varphi = \varphi_1 = \dots\dots\dots,$$

dann

$$(7) \quad T^{\alpha\beta} \rho_\alpha \rho_\beta = \bar{T}^{\alpha\beta} \rho_\alpha \rho_\beta = \dots\dots\dots$$

Besteht (7) für alle Kugeln durch \mathfrak{R} , dann muss sein

$$(8) \quad T^{\alpha\beta} = \bar{T}^{\alpha\beta} = \dots\dots\dots,$$

wenn wir $T^{\alpha\beta}$ als Funktion von einem Parameter t betrachten und

$\bar{T}^{\alpha\beta}$,, wenn t sich verändert, zu erhalten denken, dann können wir mit

$$(9) \quad T^{\alpha\beta}(t) = c_1, \quad T^{\alpha\beta}(t) = c_2, \quad \dots,$$

d.h. mit

$$\frac{dT^{\alpha\beta}(t)}{dt} = 0$$

parallele Kurven auf unseren Kreisflächen bezeichnen.

Aus (9) folgt

$$(10) \quad \cos^2 \varphi = \text{const.} \cdot (\rho_1 + \rho_2)^2.$$

Wenn $\psi = \frac{\pi}{2}$ in (10), dann

$$\rho_1 + \rho_2 = 0;$$

aus $\eta = \rho_\alpha \xi^\alpha$ ergibt sich also

$$\eta = \rho_1 (\xi^I - \xi^{II}).$$

Wenn die Kugel $\eta = \rho_1 (\xi^I + \xi^{II})$ harmonisch zu ξ^I , ξ^{II} und $\eta = \rho_1 \times (\xi^I - \xi^{II})$ ist, so ergibt sich

$$(11) \quad \cos^2 \bar{\varphi} = \text{const.} \cdot (\rho_1 - \rho_2)^2.$$

Aus (10) und (11) folgt

$$\left(\frac{\cos \varphi}{\cos \bar{\varphi}} \right)^2 = \left(\frac{\rho_1 + \rho_2}{\rho_1 - \rho_2} \right)^2,$$

d.h.

$$(12) \quad \cos \varphi : \cos \bar{\varphi} = \pm (\rho_1 + \rho_2) : (\rho_1 - \rho_2).$$

Aus (12) kann man $\bar{\varphi}$ finden, weil $(\rho_1 : \rho_2)$ und φ bekannt sind.

Ist

$$T^{\alpha\beta}(t) = f^2(t),$$

dann folgt aus (2)

$$\cos^2 \varphi = \pm f(t) (\rho_1 + \rho_2).$$

Aus (6) folgt

$$\cos^2 \varphi - \cos^2 \varphi_1 = T^{\alpha\beta} \rho_\alpha \rho_\beta - \bar{T}^{\alpha\beta} \rho_\alpha \rho_\beta,$$

d.h.

$$(13) \quad \sin(\varphi + \bar{\varphi}) \sin(\varphi - \bar{\varphi}) = 16 (\bar{T}^{\alpha\beta} - T^{\alpha\beta}).$$

Betrachten wir

$$(14) \quad \cos^2 \varphi = \frac{T^{\alpha\beta}(t) \rho_\alpha \rho_\beta}{A^{\alpha\beta}(t) \rho_\alpha \rho_\beta},$$

dann haben wir aus (14) das Ergebnis:

$$\frac{d \cos^2 \varphi}{dt} = \frac{\left(\frac{dT^{\alpha\beta}}{dt} - A^{\alpha\beta} - T^{\alpha\beta} \frac{dA^{\alpha\beta}}{dt} \right) \rho_\alpha \rho_\beta}{[A^{\alpha\beta}(t) \rho_\alpha \rho_\beta]^2}$$

Wenn

$$\frac{d \cos^2 \varphi}{dt} = 0,$$

so

$$(15) \quad \frac{d(T^{\alpha\beta})}{dt} : \frac{d(A^{\alpha\beta})}{dt} = T^{\alpha\beta} : A^{\alpha\beta}.$$

Betrachten wir

$$\eta = \rho_\alpha \xi^\alpha$$

als Kugelbüschel, dann zeigt sich (2) in der Form

$$\cos^2 \varphi = {}^{\alpha\beta} (r \rho_{1,\alpha} + t \rho_{2,\alpha}) (r \rho_{1,\beta} + t \rho_{2,\beta}),$$

d.h.

$$(16) \quad \cos^2 \varphi = r^2 T^{\alpha\beta} \rho_{1,\alpha} \rho_{1,\beta} + r t T^{\alpha\beta} (\rho_{1,\beta} \rho_{2,\alpha} + \rho_{1,\alpha} \rho_{2,\beta}) + t^2 T^{\alpha\beta} \rho_{2,\alpha} \rho_{2,\beta},$$

wobei r, t zwei Konstanten sind.

Aus (16) folgt

$$(17) \quad \cos^2 \varphi = r^2 \cos^2 \psi + r t T^{\alpha\beta} (\rho_1^\beta \rho_2^\alpha + \rho_1^\alpha \rho_2^\beta) + t^2 \cos^2 \theta,$$

wobei

$$\cos^2 \psi = T^{\alpha\beta} \rho_{1;\alpha} \rho_{1;\beta}, \quad \cos^2 \theta = T^{\alpha\beta} \rho_{2;\alpha} \rho_{2;\beta}$$

ist.

Wenn φ , ψ und θ bekannt sind, dann können wir aus (17)

$$T^{\alpha\beta} (\rho_{1;\alpha} \rho_{2;\beta} + \rho_{2;\alpha} \rho_{1;\beta})$$

finden, wobei

$$(18) \quad \frac{T^{\alpha\beta} \rho_{1;\alpha} \rho_{2;\beta}}{\sqrt{(T^{\alpha\beta} \rho_{1;\alpha} \rho_{1;\beta}) (T^{\alpha\beta} \rho_{2;\alpha} \rho_{2;\beta})}} \leq 1$$

besteht.

Aus (14) entsteht

$$\cos^2 \varphi(t) = T^{\alpha\beta}(t) \rho_\alpha \rho_\beta,$$

so folgt

$$\int \cos \varphi dt = \int \sqrt{T^{\alpha\beta}(t) \rho_\alpha \rho_\beta} dt + \text{const.},$$

d.h.

$$(19) \quad \sin \varphi = \int \sqrt{T^{\alpha\beta} \rho_\alpha \rho_\beta} dt + \text{const.};$$

weiter ergeben sich

$$(20) \quad \cos \varphi = \int \sqrt{[A^{\alpha\beta}(t) - T^{\alpha\beta}(t)] \rho_\alpha \rho_\beta} dt + \text{const.},$$

$$(21) \quad \tan \varphi = \int \frac{1}{T^{\alpha\beta} \rho_\alpha \rho_\beta} dt + \text{const.},$$

$$(22) \quad \cot \varphi = - \int \frac{1}{[A^{\alpha\beta} - T^{\alpha\beta}] \rho_\alpha \rho_\beta} dt + \text{const.},$$

u. s. w.

wobei

$$A^{\alpha\beta}(t) \rho_\alpha \rho_\beta = 1$$

ist.

Angenommen, dass verschiedene Kugeln durch \mathfrak{R} mit \mathfrak{R} verschiedene Winkeln

$$\varphi, \varphi_1, \varphi_2, \dots$$

enthalten, so folgt

$$(23) \quad \begin{cases} \cos^2 \varphi = T^{\alpha\beta} \rho_0 \rho_0, \\ \cos^2 \varphi_1 = T^{\alpha\beta} \rho_1 \rho_1, \\ \cos^2 \varphi_2 = T^{\alpha\beta} \rho_2 \rho_2, \\ \dots\dots\dots \\ \dots\dots\dots \end{cases}$$

Betrachten wir hierbei

$$T^{\alpha\beta} \rho_h \rho_k = 0,$$

dann können wir setzen

$$(24) \quad \cos^2 \varphi \equiv T^{\alpha\beta} \rho_\alpha \rho_\beta = e_1^2 \cos^2 \varphi + e_2^2 \cos^2 \varphi_1 + e_3^2 \cos^2 \varphi_2 + \dots\dots + e_n^2 \cos^2 \varphi_n,$$

wobei

$$\rho_\alpha = e_1 \lambda_{0\alpha} + e_2 \lambda_{1\alpha} + \dots\dots + e_n \lambda_{n\alpha},$$

sind und e_i Konstanten bedeuten.

Betrachten wir

$$(25) \quad k^2 = T^{\alpha\beta} \rho_\alpha \rho_\beta / A^{\alpha\beta} \rho_\alpha \rho_\beta,$$

wobei

$$(26) \quad \cos^2 \varphi = k^2$$

ist. Aus (26) folgt

$$(27) \quad (T^{\alpha\beta} - k^2 A^{\alpha\beta}) \rho_\alpha \rho_\beta = 0.$$

Wenn (3) durch Büscheltransformation

$$(28) \quad \rho_\alpha = a_\alpha^k \bar{\rho}_k$$

zu

$$(29) \quad (\bar{T}^{\alpha\beta} - k^2 \bar{A}^{\alpha\beta}) \bar{\rho}_\alpha \bar{\rho}_\beta = 0$$

transformiert wird, so folgt

$$(30) \quad | T^{\alpha\beta} - k^2 \bar{A}^{\alpha\beta} = D^2 | T^{\alpha\beta} - k^2 A^{\alpha\beta} |,$$

wobei $D = |a_{\alpha}^k|$ ist.

Entwickelt man die Determinante auf der rechten Seite nach Potenzen von k , so erhält man

$$(31) \quad k^5 \varphi_3 + k^4 \varphi_2 + k^3 \varphi_1 + \varphi_0,$$

wo die φ_k Funktionen der Koeffizienten

$$T^{\alpha\beta} - k^2 A^{\alpha\beta} \quad \text{und} \quad \bar{T}^{\alpha\beta} - k^2 \bar{A}^{\alpha\beta}$$

der beiden Formen

$$(32) \quad (T^{\alpha\beta} - k^2 A^{\alpha\beta}) \rho_{\alpha} \rho'_{\beta}$$

und

$$(33) \quad (\bar{T}^{\alpha\beta} - k^2 \bar{A}^{\alpha\beta}) \rho_{\alpha} \rho_{\beta}$$

sind.

Aus (30) folgt

$$(34) \quad \varphi_{\sigma} = A^2 \varphi_{\sigma} \quad (\sigma=0, 1, 2, 3).$$

Die Funktionen $\varphi_0, \varphi_1, \varphi_2, \varphi_3$ sind daher die Invarianten (Simultaninvarianten) der beiden Formen (32) und (33).

Aus THOMSENS⁽¹⁾ und Süß⁽²⁾ Arbeiten kann man die Theorie der relativen Differentialkugelgeometrie,⁽³⁾ relativen Differentialkreisgeometrie und relativen Kugellaffingeometrie begründen.

Es sei ein Kreis \mathfrak{K} in R_2 gegeben.

$$\varphi = \rho_{\alpha} \eta^{\alpha}$$

ist eine normierte Kugel durch \mathfrak{K} , dann folgt

$$(\varphi \varphi) = 1.$$

Nehmen wir auf unserer Kreisfläche $\varphi(u^1, u^2)$ eine Kurve $u^k(t)$ an und bezeichnen die Ableitungen nach t mit δ , so genügt eine Kugel

(1) THOMSEN, G.: Über konforme Geometrie (II), Abh. aus dem Math. Seminar der Hamb. Univ. Bd. IV (1925) S. 127.

(2) SÜSS, W.: Zur relativen Differentialgeometrie, I, Japanese Journ. of Math. Vol. IV (1927) p. 57.

(3) l.c. (I) S. 140.

\Re durch drei benachbarte Punkte der Kurve den Bedingungen

$$\Re \xi = 0, \Re \delta \xi = 0, \Re \delta^2 \xi = 0.$$

Setzen wir also

$$\Re = \xi + \alpha^i \xi_i + \beta \varphi,$$

so ergibt sich nach BLASCHKE⁽¹⁾

$$\Re = \xi + \rho e_p^i \delta u^p \xi_i + \frac{g_{pq} \delta u^p \delta u^q + \rho e_{ik} \delta u^i \delta u^k}{s_{pq} \delta u^p \delta u^q} \varphi,$$

wobei $\xi(u^1, u^2)$ Envelopenmäntel von φ ist.

(6)

Mit

$$(1) \quad \xi = \xi(u^1, u^2, u^3), \quad (\xi \xi = 0, u^1 = u, u^2 = v, u^3 = w)$$

bezeichnen wir den Flächenpunkt eines dreifachorthogonalen Flächensystems im konformalen Raume.

Setzen wir

$$(2) \quad \begin{cases} a = \xi_1^2, & b = \xi_2^2, & c = \xi_3^2, \\ f = \xi_2 \cdot \xi_3, & g = \xi_3 \cdot \xi_1, & h = \xi_1 \cdot \xi_2, \end{cases}$$

dann folgt

$$\cos \lambda = \frac{f}{\sqrt{bc}}, \quad \cos \mu = \frac{g}{\sqrt{ca}}, \quad \cos \nu = \frac{h}{\sqrt{ab}},$$

wobei

$$\xi_1 = \frac{\partial \xi}{\partial u}, \quad \xi_2 = \frac{\partial \xi}{\partial v}, \quad \xi_{23} = \frac{\partial^2 \xi}{\partial u \partial w},$$

$$\lambda = \angle (\text{Richtungen von } \xi_2 \text{ und } \xi_3),$$

u.s.w..

Der Rauminhalt dV zwischen

(1) BLASCHKE, W.: Ueber knoforme Geometrie IV, Abh. aus dem Math. Seminar der Hame. Univ. IV. Bd. (1926) S. 227.

$$u, u+du, v, v+dv, w, w+dw$$

ist mit

$$dV = [\xi_1 du, \xi_2 dv, \xi_3 dw]$$

gegeben.⁽¹⁾

(7)

ξ bezeichnet eine Kugel in R_n .

Mit

$$\xi^a [a=I, II, \dots n]$$

kann man zwei Punkte in R_n bezeichnen.

Wir nennen

$$(1) \quad \xi^a = \xi^a(t) [a=I, II, \dots n]$$

ein Paar von Kurven (c) in R_n , wobei t ein Parameter ist. Als Bogenlänge s der Kurve (c) definieren wir das Integral

$$(2) \quad s = \int_{t_0}^t F(\xi^I, \xi^{II}, \dots, \xi^n; \dot{\xi}^I, \dot{\xi}^{II}, \dots, \dot{\xi}^n) dt \\ \equiv \int_{t_0}^t F(\xi, \dot{\xi}) dt.$$

Wir werden sofort beweisen, dass

$$(3) \quad \rho \equiv \frac{\partial F(\xi, \dot{\xi})}{\partial \dot{\xi}} - \frac{d}{dt} \left(\frac{\partial F(\xi, \dot{\xi})}{\partial \dot{\xi}} \right)$$

in jedem Punkte einer beliebigen Parameterkurve ein kovarianter Vektor ist, den wir den EULERSchen Vektor der Parameterkurve nennen. Man kann zeigen, dass ein Extremalbogen durch das Verschwinden des EULERSchen Vektors charakterisiert ist.

$$(4) \quad f_i(\xi^I, \xi^{II}, \dots, \xi^{(n)}) = \text{const.}, i=1, 2, \dots, n$$

(1) WEATHERBURN, C. E.: Differentialgeometry of three Dimensions (I), Cambridge (1927) p. 64.

bezeichnen n Systeme von Kurvenpaar.

Wenn zwei Kurvensysteme f_i, f_j zueinander senkrecht sind, dann folgt

$$g^{\lambda\mu} \frac{\partial f_i}{\partial x^\lambda} \frac{\partial f_j}{\partial x^\mu} = 0, \quad i \neq j.$$

Die notwendige und hinreichende Bedingung dafür, dass (4) n senkrechte Systeme von Kurvenpaar bilden, ist⁽¹⁾ die

$$(5) \quad h_{\lambda\mu} i^\lambda_a i^\mu_c i^v_c = 0$$

(8)

Wir können zwei neue Kugeln

$$(1) \quad \xi^a = \sum_{\beta=1}^{\Pi} c^a_\beta \xi^\beta \quad [a=I, II]$$

in R_s als Linearkombination der Kugeln ξ^β in R_s einführen mit Koeffizienten c^a_β , deren Determinante

$$|c^a_\beta| \neq 0$$

sein muss, wenn ξ^* und ξ^{**} nicht proportional werden sollen, und können dann ebensogut durch die ξ^a unsere Kugeln darstellen.

Wenn

$$(2) \quad \rho \xi^a = \sum_{\beta=1}^{\Pi} c^a_\beta \xi^\beta,$$

oder

$$(3) \quad \begin{cases} (c^I_1 - \rho) \xi^I + c^I_2 \xi^{II} = 0, \\ c^I_1 \xi^I + (c^I_2 - \rho) \xi^{II} = 0, \end{cases}$$

dann

$$(4) \quad D(\rho) \equiv \begin{vmatrix} c^I_1 - \rho & c^I_2 \\ c^I_1 & c^I_2 - \rho \end{vmatrix} = 0,$$

(1) EISENHART, L. P.: Riemannian Geometry, Princeton (1926) p. 118.

wobei

$$\xi^a \neq 0.$$

Aus (4) ergibt sich

$$(5) \quad D(\rho) \equiv \rho^3 - (c_1^1 + c_2^2) \rho + c_1^1 c_2^2 - c_2^1 c_1^2 = 0.$$

Für jede ρ in (5) besteht (2).

Für neue Kugeln

$$\xi^a = \sum_{\beta=1}^{\text{III}} c_{\beta}^a \xi^{\beta} \quad [a=\text{I, II, III}]$$

in R_s wird ξ^* als Linearkombinationen der Kugeln ξ^{β} in R_s mit Koeffizienten c_{β}^a eingeführt.

So besteht dasselbe in diesem Falle wie oben.

(9)

$$(1) \quad \varphi(\xi^{\text{I}}, \xi^{\text{II}}, \xi^{\text{III}}) = \text{const.}$$

bezeichnet eine Schar von Kurvenpaar in R_s . Aus (1) folgt

$$d\varphi = 0,$$

oder⁽¹⁾

$$\nabla[\lambda_1 \xi^{\text{I}} \dots \dots V_{\lambda_3}] \xi^{\text{III}} = 0,$$

oder mit

$$(2) \quad \begin{cases} \xi^{\text{I}} = \varphi(u, v), \\ \xi^{\text{II}} = \chi(u, v), \\ \xi^{\text{III}} = \psi(u, v), \end{cases}$$

kann man eine Schar von Kurvenpaar in R_s bezeichnen, wobei u, v zwei Parameter sind.

Im allgemeinen kann man mit

(1) SCHOUTEN, J. A.: Der Ricci-Kalkül, Berlin, 1924, S. 104.

$$(3) \quad \begin{cases} \xi^I = f_1(u^1, u^2, \dots, u^{n-1}), \\ \xi^{II} = f_2(u^1, u^2, \dots, u^{n-1}), \\ \dots\dots\dots \\ \xi^n = f_n(u^1, u^2, \dots, u^{n-1}), \end{cases}$$

eine Schar von Kurven in R_n bezeichnen, wobei u^i Parameter, ξ^i Kugeln in R_n sind.

Mit g_{ik} bezeichnen wir den symmetrischen positiv definiten Tensor zweiter Stufe, dann folgt

$$(4) \quad g_{ij} = g_{ji},$$

g_{ij} ist der Fundamentaltensor.

Setzen wir

$$(5) \quad g = \begin{vmatrix} g_{11} & \dots & g_{1n} \\ \dots & \dots & \dots \\ g_{n1} & \dots & g_{nn} \end{vmatrix}$$

ein, dann folgt

$$(6) \quad g = |g_{ik}| > 0.$$

Wir notieren die g^{ik} mit g^{ik} verknüpfenden tensoriellen Relationen.

$$(7) \quad g_{ik} g^{kj} = \delta_k^j,$$

wobei δ_k^i die Kroneckers deltas sind.

Es gilt

$$A^{\alpha\beta} A_{\beta\gamma} = \begin{cases} 1 & \text{für } \alpha = \gamma, \\ 0 & \text{für } \alpha \neq \gamma, \end{cases}$$

$$\frac{1}{2} A^{\alpha\beta} A_{\alpha\beta} = 1,$$

Man kann weiter untersuchen wie in meiner Arbeit.⁽¹⁾

(1) NAKAJIMA, S.: Differentialgeometrie der Hyperoblischen, Tôhoku Math. Journ. 31, (1929) p. 247.

$$(8) \quad \phi(\xi^I, \xi^{II}, \xi^{III}, c_1, c_2, \dots, c_n) = 0$$

bezeichnet ein Paar von Kurven im R_3 , wobei $\xi^I, \xi^{II}, \xi^{III}$ drei Kugeln in R_3 und c_i Parameter sind.

Aus

$$(9) \quad f_1(\xi^I, \xi^{II}, \xi^{III}) = c_1,$$

$$(10) \quad f_2(\xi^I, \xi^{II}, \xi^{III}) = c_2,$$

$$(11) \quad F(c_1, c_2) = 0,$$

folgt

$$(12) \quad F(f_1, f_2) = 0.$$

(12) bedeutet Schnittkurven von (2) und (3).

Es gelte $U=0$, die Gleichung der Schnittkurve (12), wo $U=F(f_1, f_2)$ ist, dann folgt

$$\frac{\partial U}{\partial \xi^I} = F'(f_1) \frac{\partial f_1}{\partial \xi^I} + F'(f_2) \frac{\partial f_2}{\partial \xi^I},$$

$$\frac{\partial U}{\partial \xi^{III}} = F'(f_1) \frac{\partial f_1}{\partial \xi^{III}} + F'(f_2) \frac{\partial f_2}{\partial \xi^{III}},$$

$$\frac{\partial U}{\partial \xi^{II}} = F'(f_1) \frac{\partial f_1}{\partial \xi^{II}} + F'(f_2) \frac{\partial f_2}{\partial \xi^{II}}.$$

Sollen diese drei in $F'(f_1)$ und $F'(f_2)$ linearen Gleichungen verträglich sein, so muss

$$(13) \quad \begin{vmatrix} \frac{\partial U}{\partial \xi^I} & \frac{\partial U}{\partial \xi^{II}} & \frac{\partial U}{\partial \xi^{III}} \\ \frac{\partial f_1}{\partial \xi^I} & \frac{\partial f_1}{\partial \xi^{II}} & \frac{\partial f_1}{\partial \xi^{III}} \\ \frac{\partial f_2}{\partial \xi^I} & \frac{\partial f_2}{\partial \xi^{II}} & \frac{\partial f_2}{\partial \xi^{III}} \end{vmatrix} = 0$$

sein, d.h. die Funktionaldeterminante von U, f_1, f_2 muss verschwinden.

Dieser partiellen Differentialgleichung muss also jede Gleichung

$$U=0$$

einer der Kurvenfamilie angehörigen Kurve genügen.

$\eta, \bar{\eta}, \bar{\bar{\eta}}$ bezeichnen drei Kugeln in R_3 resp. durch den Schnitt von zwei Kugeln von $\xi, \bar{\xi}; \bar{\xi}, \bar{\bar{\xi}}; \bar{\bar{\xi}}, \bar{\bar{\bar{\xi}}}$, wobei

$$(14) \quad \begin{cases} \eta = \xi + \lambda \bar{\xi}, \\ \bar{\eta} = \bar{\xi} + \lambda \bar{\bar{\xi}}, \\ \bar{\bar{\eta}} = \bar{\bar{\xi}} + \lambda \bar{\bar{\bar{\xi}}}, \end{cases}$$

sind.

Setzen wir (14) in

$$(15) \quad f(\eta, \bar{\eta}, \bar{\bar{\eta}}, t) = 0$$

ein, dann folgt

$$(16) \quad f(\xi + \lambda \bar{\xi}, \bar{\xi} + \lambda \bar{\bar{\xi}}, \bar{\bar{\xi}} + \lambda \bar{\bar{\bar{\xi}}}) = 0,$$

d.h. die Systeme von einem Kurvenpaar im R_3 , wo t ein Parameter ist.

Entwickelt man die linke Seite der Gleichung (16) nach dem Taylorschen Satze, so kommt

$$(17) \quad \begin{aligned} 0 = & f_1 + \lambda \left(\frac{\partial f_1}{\partial \eta} \bar{\xi} + \frac{\partial f_1}{\partial \bar{\eta}} \bar{\bar{\xi}} + \frac{\partial f_1}{\partial \bar{\bar{\eta}}} \bar{\bar{\bar{\xi}}} \right) + \\ & + \frac{\lambda^2}{1 \cdot 2} \left(\frac{\partial^2 f_1}{\partial \eta^2} \bar{\xi}^2 + \dots + 2 \frac{\partial^2 f_1}{\partial \eta \partial \bar{\eta}} \eta \bar{\eta} + \dots \right) \\ & + \dots + \lambda^n \lambda_2, \end{aligned}$$

wobei

$$(18) \quad \begin{aligned} f_1 & \equiv f(\xi, \bar{\xi}, \bar{\bar{\xi}}), \\ f_2 & \equiv f(\bar{\xi}, \bar{\bar{\xi}}, \bar{\bar{\bar{\xi}}}) \end{aligned}$$

sind.

Betrachten wir nur Kurvenpaar

$$(19) \quad f_1 = 0,$$

dann folgt $\lambda=0$, d.h. (16) ist im allgemeinen gleich (19).

Betrachten wir drei lineare Beziehungen

$$\begin{cases} a\xi^\lambda + \beta\eta^\lambda + \gamma\zeta^\lambda = 0, \\ a\xi^\mu + \beta\eta^\mu + \gamma\zeta^\mu = 0, \\ a\xi^\nu + \beta\eta^\nu + \gamma\zeta^\nu = 0, \quad (\lambda, \mu, \nu = 1, 2), \end{cases}$$

zwischen drei Kreisen in R_3 , dann folgt

$$\xi^{[\lambda} \eta^\mu \zeta^{\nu]} = 0,$$

wobei α, β, γ skalare Grössen sind.

(10)

Wir betrachten das Kurvensystem

$$C: \frac{d\tau}{dt} = a(t, \tau)$$

auf unserer Kreisfläche⁽¹⁾ $\theta = \theta(t, \tau)$, deren Bogenlänge s mit

$$ds^2 = \lambda [(\theta_t, \theta_t) dt^2 + 2(\theta_t, \theta_\tau) dt d\tau + (\theta_\tau, \theta_\tau) d\tau^2]$$

gegeben werden kann.

Aus der Dreiecke, die sich mit drei Seiten (τ) , $(t+dt)$, C bildet, ergibt sich

$$\begin{aligned} \frac{\sqrt{(\theta_\tau, \theta_\tau)} d\tau}{\sqrt{(\theta_t, \theta_t)} dt} &= \frac{\sqrt{(\theta_\tau, \theta_\tau)}}{\sqrt{(\theta_t, \theta_t)}} a = \frac{\sin \varphi}{\sin(\omega - \varphi)} = \\ &= \frac{\sqrt{(\theta_t, \theta_t)(\theta_\tau, \theta_\tau)}}{\sqrt{(\theta_t, \theta_t)(\theta_\tau, \theta_\tau) - (\theta_t, \theta_\tau)^2} \cot \varphi - (\theta_t, \theta_\tau)^2}, \end{aligned}$$

dann folgt

$$\begin{aligned} a &= \frac{(\theta_t, \theta_t)}{\sqrt{(\theta_t, \theta_t)(\theta_\tau, \theta_\tau) - (\theta_t, \theta_\tau)^2} \cot \varphi - (\theta_t, \theta_\tau)^2}, \\ \tan \varphi &= \frac{\sqrt{(\theta_t, \theta_t)(\theta_\tau, \theta_\tau) - (\theta_t, \theta_\tau)^2} a}{(\theta_t, \theta_t) + (\theta_t, \theta_\tau) a}, \end{aligned}$$

(1) NAKAJIMA, S.: Differentialgeometrie der Kreisscharen, (VIII) Tôhoku Math. Journ. Vol. 32 (1930) p. 214.

wobei α den Winkel zwischen c und v , ω den Winkel zwischen u und v bedeutet. Somit ergibt sich als Gleichung von c

$$\frac{d\tau}{dt} = \frac{(\theta_i, \theta_i)}{\sqrt{(\theta_i, \theta_i)(\theta_\tau, \theta_\tau) - (\theta_i, \theta_\tau)^2}} \cot \varphi - (\theta_i, \theta_\tau).$$

(11)

(1) Es sei eine allgemeine Kugelkongruenz

$$(1) \quad \xi = \xi(u^1, u^2), \quad (\xi, \xi) = 1,$$

und die beiden Envelopenmäntel davon

$$(2) \quad \begin{cases} \eta = \eta(u^1, u^2), & (\eta, \eta) = 0 \\ \zeta = \zeta(u^1, u^2), & (\zeta, \zeta) = 0 \end{cases}$$

gegeben, wobei u^1, u^2 zwei Parameter sind.

Daraus folgt

$$(3) \quad \begin{cases} \xi, \eta = 0, & \xi, \zeta = 0, & \eta, \zeta = 0, & \zeta, \zeta_k = 0, \end{cases}$$

$$(4) \quad \begin{cases} \xi, \eta_k = 0, & \xi, \zeta_k = 0, & \xi, \zeta = 0, & \xi_i = \frac{\partial \xi}{\partial u^i}. \end{cases}$$

Wir normieren die bis auf je einen skalaren Faktor bestimmten Vektoren η und ζ , indem wir zunächst

$$(5) \quad \eta, \zeta = 1$$

setzen.

Wir wollen vier linear unabhängige Vektoren η, ζ, ξ_k aufbauen und derartig setzen:

$$(6) \quad \eta_i = p_i^k \xi_k + q_i \eta.$$

Aus (3) folgt

$$(7) \quad \xi, \xi_{ik} du^k = -\xi_i \xi_k du^k.$$

Setzen wir für einen Augenblick

$$(8) \quad \xi = \alpha^i \xi_i + \beta \eta + \gamma \zeta$$

und multiplizieren (8) mit η , η_i , so ergibt sich :

$$(9) \quad \gamma = 0, \quad \alpha^i = 0,$$

also

$$(10) \quad \xi = \beta \eta$$

Setzen wir (10) in (7) ein, so erhalten wir die Gleichungen

$$(11) \quad (\beta \eta \xi_{,k} + \xi_i \xi_k) d u^i = 0.$$

Wir benutzen jetzt zur Massbestimmung auf ξ und zur Festsetzung der Tensorbezeichnung die positiv definite quadratische Grundform

$$(12) \quad d \xi^2 = \xi_i \xi_k d u^i d u^k = A_{ik} d u^i d u^k.$$

Bilden wir dann den schiefsymmetrischen Tensor E_{ik} mit

$$(13) \quad E^{ik} = \frac{1}{(A_{11} A_{22} - A_{12}^2)^{\frac{1}{2}}},$$

so ergibt sich aus (11) für β die quadratische Gleichung

$$(14) \quad E^{ik} E^{rs} (\beta \eta \xi_{ir} + A_{ir}) (\beta \eta \xi_{ks} + A_{ks}) = 0.$$

Wir wollen nun ξ durch die invariante Forderung normieren, dass ξ die Mittelstelle der Kugeln η und ξ sein soll.

Dann muss in (14) das in β lineare Glied wegfallen :

$$(15) \quad E^{ik} E^{rs} (A_{ks} \eta \xi_{ir} + A_{ir} \eta \xi_{ks}) \\ = 2 A^{ir} \eta \xi_{ir} = 0.$$

Wir haben nebenbei gefunden :

Dafür, dass das *Kugelsystem* $\xi(u^1, u^2)$ aus der Mittelstelle der η und ξ besteht, die von dem ξ umhüllt wird, ist die Bedingung (15) notwendig.

(2) Betrachten wir zwei senkrechte Kugelkongruenzen $\xi(u^1, u^2)$, $\xi(u^1, u^2)$ in R_n , dann folgt

$$(1) \quad (\xi(u^1, u^2), \xi(u^1, u^2)) = 0.$$

Sind $\xi(u^1, u^2)$ zu der benachbarten Kugel $\xi(u^1, u^2) + \delta \xi(u^1, u^2)$

senkrecht, so folgt

$$(2) \quad (\xi(u^1, u^2) + \delta \hat{\xi}(u^1, u^2), \xi(u^1, u^2)) = 0.$$

Aus (1), (2) ergibt sich

$$(3) \quad (\delta \hat{\xi}(u^1, u^2), \xi(u^1, u^2)) = 0.$$

Sind $\hat{\xi}(u^1, u^2) + \delta \hat{\xi}(u^1, u^2)$ zu $\xi(u^1, u^2) + \delta \xi(u^1, u^2)$ senkrecht, dann folgt

$$(4) \quad (\delta \hat{\xi}(u^1, u^2), d\xi(u^1, u^2)) = 0.$$

Aus (4) folgt

$$(5) \quad \frac{\partial \hat{\xi}(u^1, u^2)}{\partial u^i} \frac{\partial \xi(u^1, u^2)}{\partial u^j} \delta u^i d u^j = 0.$$

Wenn $\hat{\xi}(u^1, u^2)$ zu $\xi(u^1, u^2)$ und zur benachbarten Kugel von $\xi(u^1, u^2)$ senkrecht sind, dann folgt

$$(6) \quad (\hat{\xi}, \xi) = 0, \left(\hat{\xi}, \frac{\partial \xi}{\partial u^1} \right) = 0, \left(\hat{\xi}, \frac{\partial \xi}{\partial u^2} \right) = 0,$$

so folgt

$$(7) \quad \left(\frac{\partial \hat{\xi}}{\partial u^i}, \frac{\partial \xi}{\partial u^j} \right) + \left(\hat{\xi}, \frac{\partial^2 \xi}{\partial u^i \partial u^j} \right) = 0 \quad (i, j = 1, 2).$$

Aus (5), (7) ergibt sich

$$(8) \quad \left(\hat{\xi}, \frac{\partial^2 \xi}{\partial u^i \partial u^j} \right) \delta u^i d u^j = 0.$$

Wenn wir $\hat{\xi}$ zwischen (6), (8) auslassen, dann hat man das Ergebnis:

$$(9) \quad \left| \xi, \frac{\partial \xi}{\partial u^1}, \frac{\partial \xi}{\partial u^2}, \frac{\partial^2 \xi}{\partial u^i \partial u^j} \delta u^i d u^j \right| = 0.$$

Setzen wir

$$\delta u = d u,$$

in (9), dann folgt

$$(10) \quad \left| \xi, \frac{\partial \xi}{\partial u^1}, \frac{\partial \xi}{\partial u^2}, \frac{\partial^2 \xi}{\partial u^1 \partial u^2} du^1 du^2 \right| = 0.$$

(10) ist die Gleichung von einer Kreisfläche, die zu einer Kugel senkrecht ist.

N.B. In LIES Kugelgeometrie bestehen :

$$\xi'' = -\xi + a \eta + b \varphi + c u + \bar{c} \bar{u},$$

$$\eta' = -a \xi' + f \varphi + k u + m \bar{u},$$

$$\varphi' = -b \xi - f \eta + g u + e \bar{u},$$

$$u' = -c \xi' - k \eta - g \varphi + h \bar{u},$$

$$\bar{u} = -\bar{c} \xi' - m \eta - e \varphi - h u,$$

wo

$$(\xi \xi)_e = (\eta \eta)_e = (\varphi \varphi)_e = 1, \quad (\xi' \xi')_e = 1,$$

$$(u u)_e = (\bar{u} \bar{u})_e = 0, \quad (u \bar{u})_e = 1,$$

$$(\xi \eta)_e = (\xi \varphi)_e = (\xi u)_e = (\xi \bar{u})_e = 0,$$

$$(\xi' \eta)_e = (\xi' \varphi)_e = (\xi' u)_e = (\xi' \bar{u})_e = 0,$$

$$(\eta \varphi)_e = (\eta u)_e = (\eta \bar{u})_e = 0, \quad (u \varphi)_e = (\bar{u} \varphi)_e = 0,$$

$$d\sigma^2 = (d\xi d\xi)_e, \quad \frac{d\xi}{d\sigma} = \xi',$$

wobei $a, b, c, \bar{c}, f, g, m, e, h, k$ skalare Zahlen sind.

(12)

Mit ξ, η sei das Quintupel der pentasphärischen Koordinaten eines Punktes bezeichnet und mit ξ^2, η^2 die quadratischen Formen, die die linken Seiten der Bedingungsgleichungen $\xi^2=0, \eta^2=0$ zwischen diesen homogenen überzähligen Punktkoordinaten darstellen.

Es seien nun $\xi(u^1, u^2), \eta(u^1, u^2)$ zwei Flächen, u^k das Parameter auf ihnen, $\wp(u^1, u^2)$ ein System von Kugeln, das diese Flächen umhüllt, so dass die Beziehungen bestehen :

$$(1) \quad \begin{cases} x^2=0, \quad x \, p=0, \quad x_i \, p=0, \quad x^i=\frac{\partial x}{\partial u^i}; \\ y^2=0, \quad y \, p=0, \quad y_i \, p=0, \quad y_i=\frac{\partial y}{\partial u^i}. \end{cases}$$

Wir können die homogenen Koordinaten so normieren, dass

$$(2) \quad x \, y=1, \quad p^2=1$$

wird.

Die quadratischen Formen

$$(3) \quad \begin{cases} (\partial x^2 - (x_i \delta u^i)(x_k \delta u^k) = g_{ik} \delta u^i \delta u^k, \quad g_{ik} = x_i x_k, \\ (\partial y^2 - (y_i \delta u^i)(y_k \delta u^k) = G_{ik} \delta u^i \delta u^k, \quad G_{ik} = y_i y_k \end{cases}$$

sind positiv definit und

$$(4) \quad \begin{cases} (x \, x_1 \, x_2 \, p \, y)^2 = - \begin{vmatrix} 0 & 0 & 0 & 0 & 1 \\ 0 & g_{11} & g_{12} & 0 & 0 \\ 0 & g_{21} & g_{22} & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 \end{vmatrix} = \begin{vmatrix} g_{11} & g_{12} \\ g_{21} & g_{22} \end{vmatrix}, \\ (y \, y_1 \, y_2 \, p \, x)^2 = \begin{vmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{vmatrix}. \end{cases}$$

Wegen der linearen Unabhängigkeit von $x, x_k, p, y; y, y_k, p, x$ können wir

$$(5) \quad \begin{cases} x_{ik} = r_{ik} x + s_{ik} p - g_{ik} y, \\ y_{ik} = R_{ik} y + S_{ik} p - G_{ik} x \end{cases}$$

setzen.

Darin ist

$$(6) \quad x_{ik} x_i = 0, \quad y_{ik} y_i = 0$$

und

$$(7) \quad \begin{cases} g_{ik} = \xi_i \xi_k = -\xi_k \xi_i, \\ G_{ik} = \eta_i \eta_k = -\eta_k \eta_i, \\ r_{ik} = \xi_i \eta_k = -\xi_k \eta_i = -\eta_k \xi_i, \\ R_{ik} = \eta_i \xi_k = -\eta_k \xi_i = -\xi_k \eta_i, \\ s_{ik} = \xi_i p_k = -\xi_k p_i = -\xi_k p_i, \\ S_{ik} = \eta_i p_k = -\eta_k p_i = -\eta_k p_i. \end{cases}$$

Nehmen wir auf unserer Fläche (\mathfrak{r}) eine Kurve $u^k(t)$ an und bezeichnen die Ableitungen nach t mit δ , so genügt eine Kugel \mathfrak{R} durch drei benachbarte Punkte der Kurve den Bedingungen

$$(8) \quad \mathfrak{R} \xi = 0, \quad \mathfrak{R} \delta \xi = 0, \quad \mathfrak{R} \delta^2 \xi = 0.$$

Setzen wir also

$$(9) \quad \begin{cases} \mathfrak{R} = \xi + \alpha^i \xi_i + \beta p + \gamma \eta, \\ K = \eta + A^k \eta_k + B p + I' \xi, \end{cases}$$

so folgt

$$(10) \quad (\mathfrak{R} K) = 1 - \alpha^i A^k \gamma_{ik} + \gamma I' = 0,$$

weil (1), (2), (6), (7) bestehen, wobei

$$\alpha^i = \rho e_p^i \delta u^p$$

ist, also folgt der

Satz: Wenn $1 + \gamma I' = \alpha^i A^k \gamma_{ik}$ ist, dann sind zwei Kugeln \mathfrak{R} und K zueinander senkrecht.

(13)

Mit

$$(1) \quad \varphi = a_i \xi^i + a_{II} \xi^{II} + a_{III} \xi^{III}, \quad \varphi = \bar{a}_i \bar{\xi}^i + \bar{a}_{II} \bar{\xi}^{II} + \bar{a}_{III} \bar{\xi}^{III},$$

(a_i, \bar{a}_i skalar!)

kann man zwei Geraden in R_s als Schnitt von drei Kugeln bezeichnen, wobei $\xi, \bar{\xi}$ die Kugeln in R_s sind.

Die Bedingung dafür, dass zwei Geraden $\varphi, \bar{\varphi}$ überdecken, ist die:

$$(2) \quad (\varphi \bar{\varphi}) = 0,$$

d.h.

$$(3) \quad (\xi^{\alpha} \bar{\xi}^{\lambda}) = 0.$$

Wenn sich ξ^{α} transformieren bei den Büscheltransformationen

$$(4) \quad \xi^{\alpha} = c_{\beta}^{\alpha} \xi^{\beta}, \quad |c_{\beta}^{\alpha}| \neq 0,$$

dann folgt

$$(5) \quad A^{\alpha\beta} = C_{\gamma}^{\alpha} C_{\delta}^{\beta} \bar{A}^{\gamma\delta}.$$

Wegen $A^{\alpha\beta} = A^{\beta\alpha}$ bilden $A^{\alpha\beta}$ einen symmetrischen Tensor zweiter Stufe.

Wenn eine Gerade φ auf einer Fläche $g(u^1, u^2)$ liegt, dann folgt

$$(6) \quad (\varphi g) = 0,$$

wobei u^i Parameter sind.

Ist $\varphi(u^1, u^2)$ Envelopengeradlinienfläche von $\bar{\varphi}(u^1, u^2)$, dann folgt

$$(7) \quad \left(\varphi \frac{\partial \bar{\varphi}}{\partial u^i} \right) = 0,$$

d.h.

$$(8) \quad \rho_{\alpha} \bar{\rho}_{\lambda} D^{\alpha\lambda} = 0$$

wobei

$$(9) \quad D^{\alpha\lambda} = (\xi^{\alpha} \bar{\xi}^{\lambda}), \quad \varphi = \rho_{\alpha} \xi^{\alpha}, \quad \bar{\varphi} = \bar{\rho}_{\alpha} \bar{\xi}^{\alpha}$$

ist.

Wenn

$$(10) \quad S^{\alpha\lambda} = (\xi^{\alpha} \bar{\xi}^{\lambda})$$

ein gemischter Tensor ist, dann wird er durch (4) transformiert

$$(11) \quad \tilde{S}^{\alpha\lambda} = c_{\mu}^{\alpha} \bar{c}_{\mu}^{\lambda} S^{\mu\alpha}.$$

Wir schliessen nun den Fall aus, dass die Matrix

$$(12) \quad || \xi^I, \xi^{\pi}, \xi^{\text{III}}, \bar{\xi}^I, \bar{\xi}^{\pi}, \bar{\xi}^{\text{III}} || \equiv 0$$

ist, in der *eine lineare Beziehung zwischen $\xi, \bar{\xi}$ besteht*, d.h.

$$\sigma_{\alpha} \xi^{\alpha} = \bar{\sigma}_{\lambda} \bar{\xi}^{\lambda}.$$

Vergleichen wir die beiden Arbeiten von THOMSEN⁽¹⁾ und SÜSS,⁽²⁾ so kann man die Fundamentalsätze über relative Differentialkugelgeometrie begründen.

In LIES höherer Kreisgeometrie bestehen die gleichen Beziehungen wie in meiner Arbeit.⁽³⁾

N.B. (I) Es sei eine lineare Kongruenz in LIES Kugelgeometrie

$$(\varphi \hat{\varepsilon})_0 = 0 \quad ((\varphi(u^1, u^2) \varphi(u^1, u^2))_0 = 1)$$

und die beiden envelopen K.—Kugeln

$$(\mathfrak{K} \hat{\varepsilon})_0 = 0 \quad ((\mathfrak{K} \mathfrak{K})_0 = 0),$$

$$(\bar{\mathfrak{K}} \hat{\varepsilon})_0 = 0 \quad ((\bar{\mathfrak{K}} \mathfrak{K})_0 = 0)$$

vorgegeben.

Man führe die Bezeichnung

$$\frac{\sigma \varphi}{\partial u^1} = \varphi_1 \quad \text{u.s.w.}$$

ein.

Nun setzen wir

$$(\mathfrak{K} \bar{\varepsilon})_0 = 1.$$

Legt man die folgenden Bezeichnungen und Formeln zu Grunde:

- (1) THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ. Bd. IV (1925) S. 127.
- (2) SÜSS, W.: Zur relativen Differentialgeometrie, I, Japanese Journ. of Math. Vol. IV (1927) p. 57.
- (3) NAKAJIMA, S.: Kugelgeometrie von Möbius, Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ., Vol. II (1929) p. 6.

$$(\varphi_h \varphi_k) d u^h d u^k = G_{hk} d u^h d u^k$$

(Grundform der Tensorrechnung)

$$G = G_{11} G_{22} - G_{12}^2, \quad D_{hk} = -(\varphi_h \xi_k) = (\varphi \xi_{hk}) = (\xi \varphi_{hk}),$$

$$D_{hk} = -(\varphi_h \bar{\xi}_k) = (\varphi \bar{\xi}_{hk}) = (\bar{\xi} \varphi_{hk}),$$

$$\epsilon^{11} = 0, \quad \epsilon^{12} = G^{-\frac{1}{2}} = -\epsilon^{21}, \quad \epsilon^{22} = 0,$$

$$G^{hk} = \frac{\partial}{\partial G_{hk}} \log G, \quad N_h = (\xi \bar{\xi}_h) = -(\bar{\xi} \xi_h),$$

$$D \equiv D_{11} D_{22} - D_{12}^2, \quad \bar{D} = \bar{D}_{11} \bar{D}_{22} - \bar{D}_{12}^2,$$

dann entstehen die folgenden Ableitungsgleichungen:

$$\varphi_{hk} = -G_{hk} \varphi + D_{hk} \xi + D_{hk} \bar{\xi},$$

$$\xi_h = -D_h^i \varphi_i - N_h \xi,$$

$$\bar{\xi}_h = -\bar{D}_h^i \varphi_i + N_h \bar{\xi}.$$

Die Integrabilitätsbedingungen dieser Differentialgleichungen sind

$$\mathfrak{R}^i = 1 - \epsilon^{ih} \epsilon^{kl} D_{hk} D_{li} = 1 + \frac{D}{G} D_{rs} D^{rs},$$

$$\epsilon^{kl} \bar{D}^{rp} D_{rkl} = -\epsilon^{kl} D^{rp} D_{rkl},$$

$$-\epsilon^{kl} D_k^i \bar{D}_{il} = -\epsilon^{kp} (D_{hpk l} D^{hl} + D_{hpk} D_l^{hl}).$$

(II) Hirakawa⁽¹⁾ führt seinen dritten Satz auf den auch an sich interessanten Satz 4 zurück:

Wenn alle Durchmesser einer Kurve konstanter Breite den Flächeninhalt halbieren, so ist die Kurve ein Kreis.

Diesen Satz kann man auch folgendermassen beweisen:

Es sei AB ein fester und XY ein variabler Durchmesser, Schnittpunkt beider sei P, ihr Winkel u .

Für $u \rightarrow 0$ ist sicher $AP = BP$.

Denn für alle Werte u ist der Flächensektor APX dem Sektor

(1) HIRAKAWA, J.: On a Characteristic Property of the Circle, Tôhoku Math. Journ. Vol. 37 (1933) p. 175.

BPY flächengleich nach der Voraussetzung.

In der Grenze wird dieser Inhalt aber

$$AP^2 du = BP^2 du.$$

Man kann das auch in der bekannten Weise sagen :

Der momentane Drehpunkt des halbierenden Durchmessers ist sein Mittelpunkt.

Es ist also

$$AP = BP = b = \text{Breite} = \text{const. für } u \rightarrow 0.$$

Für $u > 0$ aber schliessen wir :

Ist r der Radiusvektor von P aus, so ist

$$\int_v^w r^2 du = \int_{v+\pi}^{w+\pi} r^2 du \quad \text{für alle } v \text{ und } w.$$

Also ist

$$r^2(w+\pi) - r^2(w) = r^2(v+\pi) - r^2(v)$$

für alle v, w und insbesondere für $v=0, w=\pi$:

$$r^2(2\pi) - r^2(\pi) = r^2(\pi) - r^2(0),$$

wegen

$$r(2\pi) = r(0)$$

also

$$r(0) = r(\pi),$$

d.h.

$$AP = BP = b/2.$$

XY schneidet also AB stets im Mittelpunkt.⁽¹⁾

Da AB auch willkürlich war, so folgt diese Behauptung sofort.

(1) Vergl. NAKAJIMA, S.: On Ovals, Tôkyô Butsurigakko Zasshi, 34 (1925) p. 82.

Studies on Concentrating the Hydrogen Isotope H^2 by the Electolysis of Water. Part I.

(With 2 Plates and 5 Text-Figures)

Yoritsune OTA

(Received for publication, February 10, 1934.)

Introduction

The first quantitative evidence for the existence of a hydrogen isotope of mass 2, was secured by UREY, BRICKWEDDE and MURPHY⁽¹⁾ and they gave for the abundance ratio of H^2 to H^1 in natural hydrogen, the value $H^2 : H^1 = 1 : 4000$. These results were obtained by an observation along Balmer lines. Since then many investigations have been conducted to determine the true value of the abundance ratio in natural hydrogen. The most provable one of value at present is the one recently obtained by BLEAKNEY and GOULD:⁽²⁾ $H^2 : H^1 = 1 : 5000$.

In spite of the very small amount of H^2 present in ordinary hydrogen, it is very promising to fractionate H^2 and H^1 completely, because the mass of H^2 is twice as large as that of H^1 and therefore the difference of properties between them is expected to be far greater than between any other pair of isotopes. UREY, BRICKWEDDE and MURPHY⁽¹⁾ first obtained a sample which contained H^2 about five times as great as natural hydrogen by evaporating liquid hydrogen at a pressure which was only a few millimeters above the triple point. Soon after this work the possibility of fractionating H^2 and H^1 by the electrolysis of water was suggested by WASHBURN and UREY.⁽³⁾ According to their theory, in the process of electrolysis of water there are two causes for excluding one isotope from the other : (1) the effect of a possible little difference between the normal

electrode potentials of H^1 and H^2 (2) the effect due to the diffusion process of two species of ions and also discharged ions in the mechanism of formation of hydrogen molecules at the cathode. And they confirmed their expectation by finding the definite increase in the abundance ratio in the residual solution obtained by the electrolysis of water to produce oxygen for industrial purposes. By virtue of this method brilliant success in fractionating H^2 and H^1 to a considerable degree was recently achieved by LEWIS and MACDONALD.⁽⁴⁾ In their experiment, water was made conductive by adding NaOH and nickel plates were used as electrodes. The solution was carefully cooled to avoid the loss of water caused by evaporation. Reducing twenty liters of water obtained from the industrial electrolytic cell used about four years to one-half of cubic centimeter by electrolysis, they could obtain water which has a specific gravity of 1.073 and contained H^2 and H^1 in ratio: $H^2 : H^1 = 2 : 1$. From their results they also estimated the ratio of the percentage loss of H^2 to that of H^1 at 0.20. Another important work of research is that of WASHBURN, SMITH and FRANDSEN.⁽⁵⁾ In their experiment, water having an initial specific gravity of 1.000034 was made conductive by adding sulphuric acid, and the solution (0.01 N) was electrolyzed by using an anode of right platinum and a gold-plated copper cathode covered with platinum black. They measured the variation of the density of water caused by continued electrolysis, and gave curves illustrating the efficiency of the fractionation.

Since it is very provable that H^2 may offer very powerful aids to researches in all branches of physics and chemistry, especially in nuclear physics, our laboratory immediately took up the study on the electrolysis method, soon after the announcement of the success of LEWIS and MACDONALD arrived here. In the following papers, the experiments and results obtained are given.

Methods and Results of the Experiment.

In order to determine the abundance ratio of H^2 to H^1 in water, the following three methods will be used: (1) Density measurement

(2) Positive ray method (3) Spectroscopic method. In the present experiment, the spectroscopic method was adopted, and the abundance ratio was determined by comparing the intensities of the H_α lines of H^1 and H^3 with each other.

According to the theory of line spectra, the position of the H^3 Balmer lines is to be shifted a little to the violet side of the corresponding H^1 Balmer lines. These separations were first measured by UREY, BRICKWEDDE and MURPHY.⁽¹⁾ They photographed the spectrum of light from a Wood hydrogen discharge tube in the second order of a 21 foot grating having dispersion of 1.3 Å. per mm., and obtained the following results.

	H_α	H_β	H_γ	H_δ
separations in A.U.	1.791	1.313	1.176	1.088

Moreover, besides the above small separated distance between the Balmer lines of H^1 and H^3 , the broadening of H^1 lines caused by over exposure cannot be avoided, therefore a spectrograph having large dispersion and resolving power must be used to detect the H^3 line from the H^1 line. The author therefore used a large glass prism

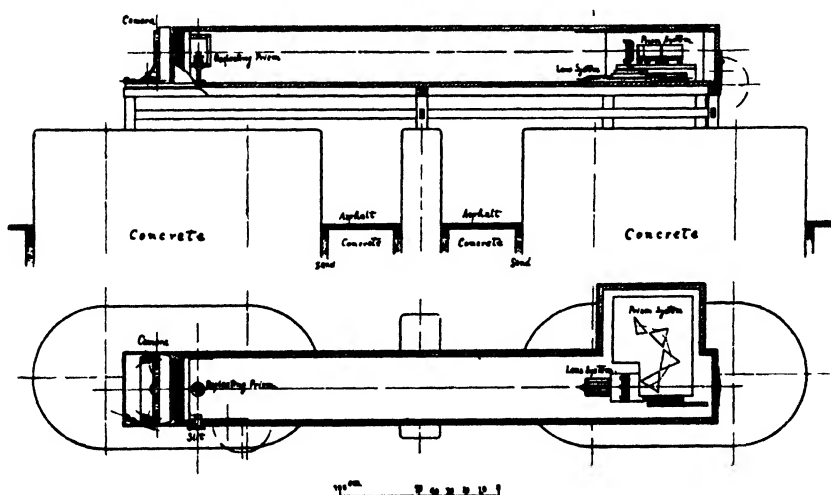


Fig. 1.

spectrograph of Littrow mounting constructed in our laboratory. The details of the design are shown in Fig. 1.

The optical train consists of one achromatic lens of 10 cm. aperture and 300 cm. focal length made by Hilger & Co. (No. E 263), a 60° prism and a 30° prism each of which is 13.0 cm. length of face \times 7.6 cm. in height by Hilger & Co. (No. E 245) and two 60° prism by Kōgaku Kōgyō & Co., which are similar in size to the above ones.

The slit is Hilger's No. F 31. The dark slide is Hilger's No. E 295.

The light that enters through the slit is reflected along the camera case by a right-angled prism of 1.4 cm. length of reflecting face \times 1 cm. in height, is collimated by the lens; passes through the train of the prisms; is reflected back by the 30° prism the reflecting face of which is coated with tin-mercury amalgam; and retraces its path through the lens, an image of the spectrum being focussed on the photographic plate. The prism system is placed on a stand

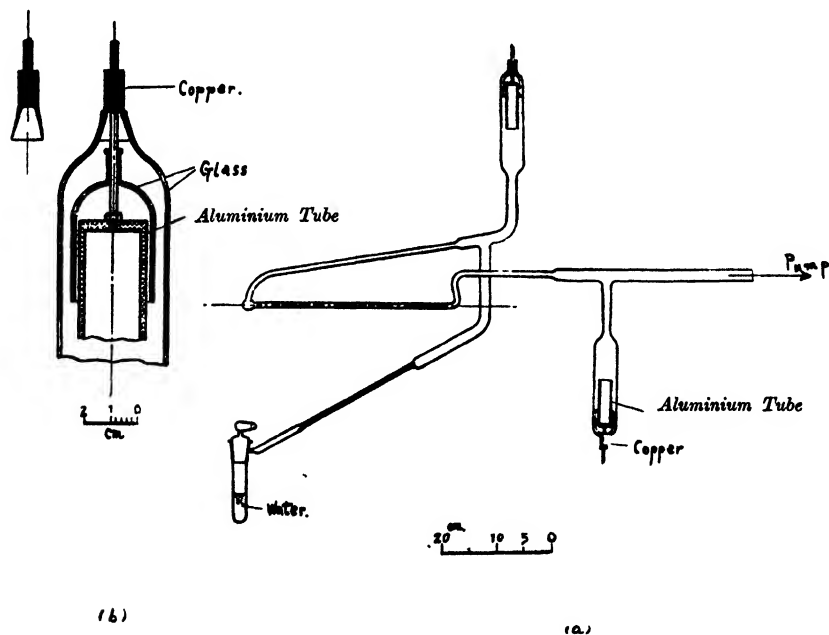


Fig. 2.

fixed to the bottom of a case of the spectrograph. The lens is mounted on a carriage which can be moved along a slide by a screw, its position being defined by a scale and an index, and its position can be accurately adjusted for the focusing purpose. The case is made of well seasoned Japan cypress. The spectrograph is settled on concrete bases separated by a layer of sand from the ground floor to avoid the disturbance caused by the shock.

This spectrograph gives a very fine image of the spectrum. The dispersion is 1.4 Å./mm. at the H_β line, so the separation of H_β^1 and H_β^2 lines on a photographic plate is about 1 mm.

The discharge tube used is the type designed by R. W. WOOD and shown in Fig. 2.

The main part of the tube is 0.8 cm. in inner diameter. Aluminium cylinders of 2.5 cm. in diameter and 10 cm. in length are used as electrodes, and each of them is fixed to a copper lead joined to a glass tube of 4 cm. in diameter as shown in Fig. 2b. The discharge tube is connected through a capillary to a small vessel in which samples of water are put in, and also to a Cenco Hyvac pump as shown in Fig. 2a.

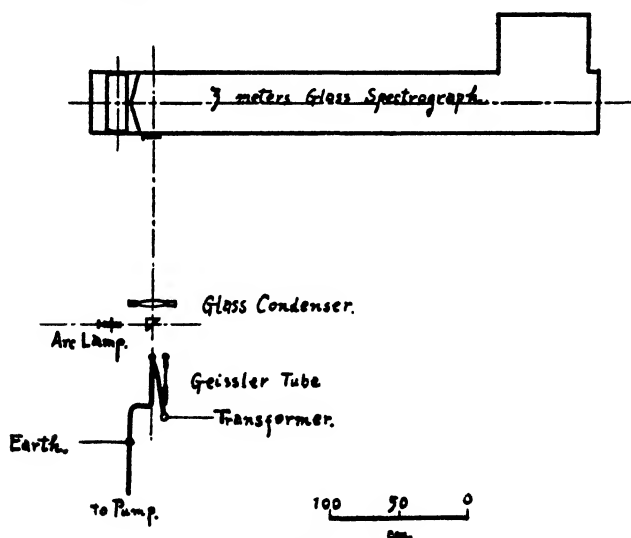


Fig. 3.

The tube is excited by a current of about 80 ma., using a 5 kw. X-ray transformer with one pole earthed. During the excitation, water vapour was continuously run through the tube.

The whole arrangement for the spectroscopic work is shown in Fig. 3.

The photographic plate used was the Ilford special rapid panchromatic plate (thin glass).

The emission of the discharge tube gave very pure atomic spectrum of hydrogen and was so intense that the image of the H^1_β line could be clearly detected on the plate by 5 seconds' exposure.

To concentrate H^3 by the electrolysis of water the following method was first tried. Fifteen liters of ordinary distilled water was made conductive by adding 5% NaOH, and was poured into twenty cells, which were provided with electrodes of iron plates and connected in series in a D.C. 100 volts circuit. The cells were cooled from the out-side by water. The current of electrolysis was about 6 amperes. The electrolysis of water was continued, collecting the solution in the smaller number of cells when it reduced in quantity, and distilling the solution when the concentration of NaOH in it rose too high to be fit to electrolyze.

From time to time the solution was examined spectrographically. In this way we could attain one of the solutions the photograph of which a faint but clear image of a new line in the expected position of the H^3_β line after two hours' exposures. Prolonging the time of exposure to 6.5 hours a plate with which the wave-length of the new line could be accurately measured, was successfully obtained. The wave-length of the new line was found to be $\lambda_{air} = 4860.03$ A.U. Comparing this value with that obtained by UREY, BRICKWEDDE and MURPHY, it was decided that this new line was really the H^3_β line. Judging from the fact that the 8 seconds' exposure is necessary to obtain the image of the H^1_β line in equal intensity with that of the H^3_β line obtained by the 6.5 hours' exposure, it was estimated that the abundance ratio in the solution was about $H^3 : H^1 = 1 : 2700$. By rough estimation it became clear that the efficiency of fractionation

was worse than that of LEWIS and MACDONALD's method.

Next, to improve the efficiency of fractionation the following method was tried. The electrolyser used was the one shown in Fig. 4.

An outer glass tube (a) was 3 cm. in inner diameter and 35 cm. in length. A hole of about 1 cm. in diameter was made on the upper part of the outer tube. An inner glass tube (b) was 10 mm. in diameter and was so constructed as to cool the solution by the stream of cooling water through it. The electrodes were made by rolling a sheet of iron shown in Fig. 4b in a cylindrical form, and they were placed in contact with an outer tube and an inner one respectively. The capacity of the electrolyser was about 100 cc.

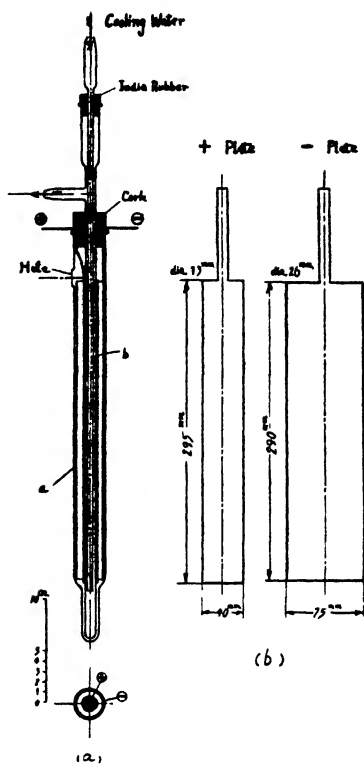


Fig. 4.

A certain great quantity of distilled water can be reduced to a small quantity of 30 cc. by this arrangement of electrolysis in the following way: 100 cc. of the distilled water is at first made conductive by resolving 10 g. of NaOH and then introduced into the cell; then the cell was operated upon with a current of 8 amperes being supplied with the distilled water through the hole twice a day till the volume reached its initial 100 cc. In such a way the solution was reduced to the quantity of 30 cc.

The solution was so well cooled by this arrangement that the decrease of its volume was almost equal to that expected by the law of electrolysis.

To know the efficiency of the fractionation, 280 cc. of distilled

water, the relative abundance being $H^2 : H^1 = 1 : 2700$, was reduced to the solution of 35 cc. in quantities. This solution was examined spectrographically and a fine photograph of the H^2 line could be obtained by 40 minutes' exposure. The relative abundance was estimated at about the value $H^2 : H^1 = 1 : 400$.

Some of the photographs taken with this solution are shown in Plate I and II. Plate Ia is a reproduction of a plate obtained by two hours' exposure, as comparison spectra the Fe and Ni spectra of a Pfund arc and also the spectra of the hydrogen discharge tube being taken on the plate. An enlarged reproduction of the plate is given in Plate Ib. Plate IIa is a reproduction of a plate obtained by 5 hours' exposure, and an enlarged one is given in Plate IIb.

To estimate the efficiency of fractionation of H^2 and H^1 , the quantity of water contained in the final solution must be known and it was found to be about 30 cc. Therefore the value of

$$\frac{R}{R_0} : \frac{V_0}{V}$$

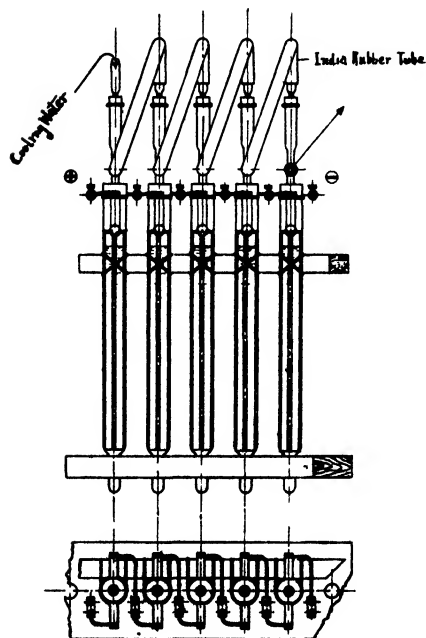


Fig. 5 .

was about 0.7, where R_0 and R , indicating the initial and final value of the abundance ratio $H^2 : H^1$, and V_0 and V , that of the volume of water. Thus the efficiency of fractionation was nearly as good as that of LEWIS and MACDONALD.

By connecting the cells in such a way as shown in Fig. 5, they are conveniently used to concentrate H^2 .

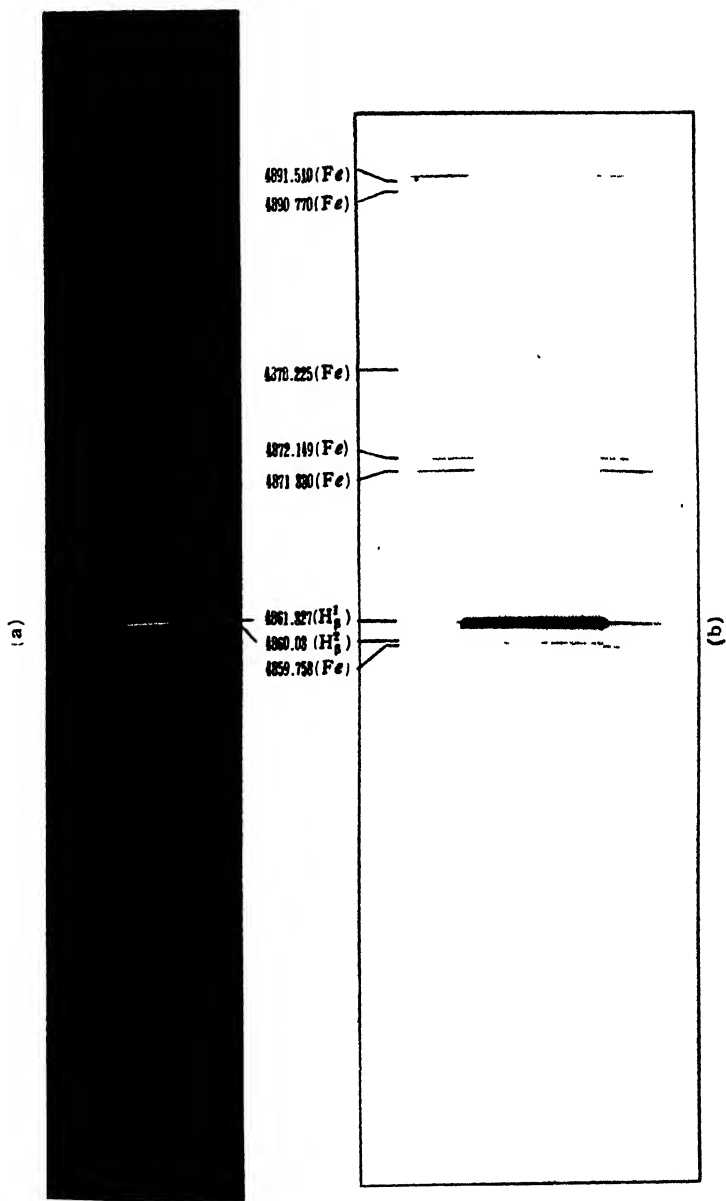
To operate them with a current of 8 amperes, about 45 of them were connected in series in a D.C. 120 volts circuit. In

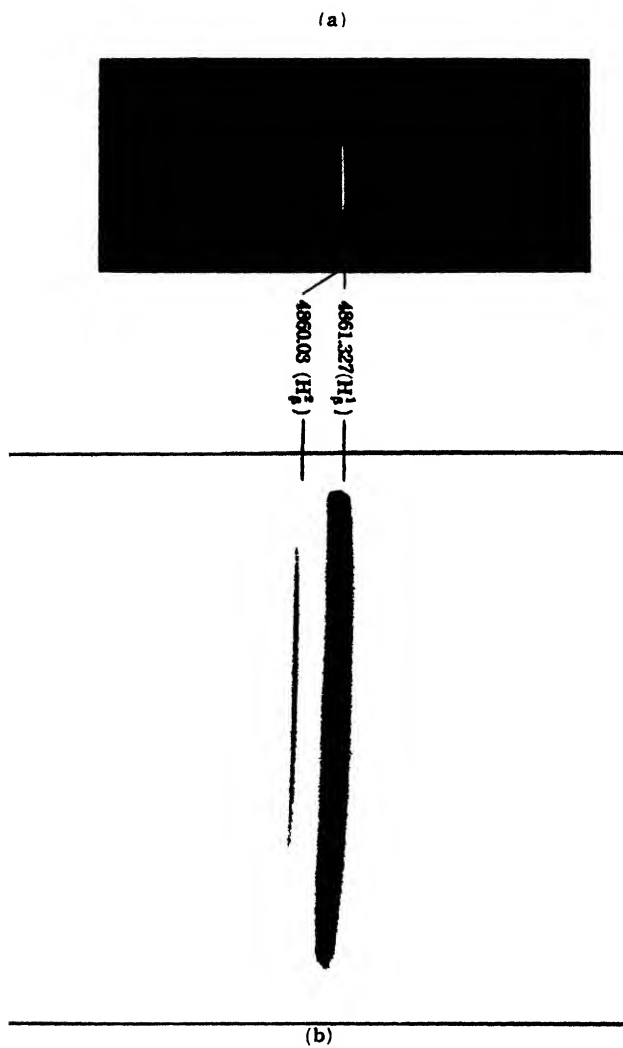
our laboratory, with these devices the experiments on the subject are being continued, the details of which will be published shortly.

The writer wishes to express his hearty thanks to Prof. B. ARAKATSU for his encouragement and invaluable advice given to this work and to Mr. Y. UEMURA for his kind help in the construction of the spectrograph.

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Notes on the Validity of the Principle of the Conservation of Spin Angular Momentum in the Process of the Artificial Disintegration of Lithium Atoms.

B. ARAKATSU

(Received for publication, February 10, 1934.)

The validity of the principle of the conservation of angular momentum was previously discussed by the present writer⁽¹⁾ and Y. OTA for various cases of the interaction processes among molecules, atoms, electrons and photons. It was pointed out that the principle does not literally hold in the case of the collision process between atom and electron, and that it is somewhat difficult to accept in the case of the radiation process $\Delta j=0$ without making some necessary modification of the fundamental conception of the word "angular momentum."

The failure of this principle was also recently shown by DÖPEL⁽²⁾ and GAILER in the case of the excitation of Hg atoms bombarded by the canal ray of He atoms.

We are now going further to say that the angular momentum does not strictly be conserved, not only in the natural process taken by the system of outer configuration, but also in the process taken by the system of nuclear configuration of atoms.

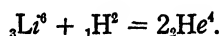
Consider the case of the artificial disintegration of Li^7 atom shot by a proton of high speed. With COCKROFT and WALTON, the discoverers of the phenomena, all of the later investigators⁽³⁾ agree in interpreting the phenomena as the nuclear "chemical" process which is appropriately expressed

by ${}_3Li^7 + {}_1H^1 = 2{}_2He^4,$

because the mass-energy equation

Mass of Li^7 + Mass of H^1 + K.E. of $\text{H}^1 = 2$. (Mass of He^4 + K.E. of He^4) is satisfactorily compatible with their careful experiments. If we now take the nuclear spinmoment of each of the colliding particles and the resulting ones in consideration and attempt to set a conservation equation of this quantity, we see, at once, that a quantum or two of the nuclear moment is annihilated by this chemical process, since the nuclear moment of proton, He and Li^7 is believed to be $\frac{1}{2}$, 0, and $\frac{3}{2}^{(4)}$ quantum respectively and so $\frac{3}{2} \pm \frac{1}{2}$ had to be anticipated to become 2 or 1, while it gave rise in observations to 0 + 0.

For the case of the disintegration of Lithium by the bombardment of atoms of the heavier isotope of hydrogen, it is stated^(3b) that the phenomena are to be expressed, as in the former case, by the "Chemical equation"



Though the experimental determination of the nuclear spin of Li^6 is not yet decisively clear, it is estimated, by some writers, from SCHÜLER's observation⁽⁴⁾ of the hyperfine structure of the spectral line of this atom, to be 0. For H^2 , the nuclear moment is determined by LEWIS and ASHLEY⁽⁴⁾ to be 1.*

Taking these values, we see also that the conservation of the nuclear moment fails in the process of the artificial disintegration by the collision of two nuclei, and namely that the apparent annihilation of one quantum moment is accompanied.

If, in both cases, the chemical process be always accompanied by the emission of a photon (γ -ray), which carries a quantum of spin

Recent experiments of FRISCH, ESTERMANN and STERN⁽⁴⁾ show that, while the mechanical moment of proton is $\frac{1}{2}$, the magnetic moment is about 2.5 and the magnetic moment of the nucleus of the heavy isotope of hydrogen is exceedingly small compared with that of proton. From these facts, we may assume that the mechanical moment of Li^6 many probably be 1, even though the magnetic moment is vanishingly small (SCHÜLER's observation). If we presumably take this value, we see at once that the conservation principle may hold well in this case. Since, however, the necessary observation of band spectra of $(\text{Li}^6)_2$ has not yet been successful notwithstanding the endeavours of many investigators,⁽⁴⁾ it is assumed, in the present paper, that the mechanical moment of Li^6 to be 0 and that of H^2 to be 1.

angular momentum $\frac{h}{2\pi}$, the total amount of the angular momentum may be conserved. Decisive evidence on this point,⁽³⁾ however, is not as yet obtainable by experiments.

At any rate the nuclear angular momentum seems not always to conserve itself in the nuclear "chemical process," but, in some cases, a quantum of this quantity creates or annihilates itself in the nuclear configuration during the process.

The alternative interpretation may be speculatively done by taking at once the angular momentum of the outer configuration of atoms just before and after the chemical reaction in account together with the nuclear one. But this is to be reserved until experimental facts are found, to justify the presumption of the existence of such an interaction between the nuclear configuration and the outer one as it is under the control of their conserving spin moments.

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BEITRÄGE ZUR GEOMETRIE DER KREISE UND KUGELN (IX):

Einige Anwendungen der Kreis- und Kugelgeometrie

SÔJI MATSUMURA

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(1) Zur relativen Differentialgeometrie und zur gewöhnlichen Kurve

Es seien $(\hat{\zeta})$, $((\hat{\zeta}\hat{\zeta})=1)$ die tetrazyklischen Kreiskoordinaten der Kreisscharen

$$(1) \quad \xi = \xi(\sigma), \quad ((\xi\xi)=1, d\sigma^2 = (d\xi d\xi))$$

und

$$(2) \quad (b) \quad ((bb)=0),$$

$$(\bar{b}) \quad ((\bar{b}\bar{b})=0)$$

die kreisscharentheoretisch normierten tetrazyklischen Punktkoordinaten des Enveloppenmantels.⁽¹⁾

Dann ist nach der SCHWARZschen Ungleichung für eine einfachgeschlossene Kurve:

$$\begin{aligned} (3) \quad L^2 &= (\int dt)^2 \leq (\int d\sigma) \left\{ \int \left(\frac{dt}{d\sigma} \right)^2 d\sigma \right\} \\ &= 4\pi \int \frac{\rho}{2} d\sigma \\ &= 4\pi S, \end{aligned}$$

wo

$$\begin{aligned} (4) \quad S &= \int \frac{\rho d\sigma}{2}, \quad dt = \text{Bogenelement von } (b), \\ d\sigma &= \text{Kontingenzwinkel von } (\xi) \\ \frac{1}{\rho} &= -\frac{d\rho}{dt} = \text{Krümmung} \end{aligned}$$

sind.

Weiter erhalten wir gleiche Beziehungen für \bar{b} , wenn \bar{b} eine einfachgeschlossene Kurve ist.

Betrachten wir Relativgeometrie der Eilinen, so folgt

$$(5) \quad \frac{dt}{d't} = \frac{\rho(b)}{\rho(e)} = \frac{(\xi'_t \xi_t)}{(\xi_t \xi_t)} = \Gamma = \frac{(\xi'_t \xi_t) d\sigma}{(\xi_t \xi_t) d'\sigma},$$

wo Γ R-Krümmungsradius von b ist und e Eicheilinie und t, σ, ξ mit t', σ' bzw. ξ' bezeichnet sind.⁽²⁾

Für den R-Umfang F von b aber gilt:

- (1) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 126.
- (2) SÜSS, W.: Zur relativen Differentialgeometrie, I, Japanese Journ. of Math. Vol. IV (1927) p. 57.

$$(6) \quad F = \oint dt = \int \Gamma d't = r d't,$$

wo $r = p/q$; p, q beide Stützfunktionen von b und c sind. Liegt die Eilinie \bar{b} ganz oder teilweise innerhalb b , so ist

$$(7) \quad F(\bar{b}) < F(b).$$

Nach meiner Arbeit folgt

$$(8) \quad \tan \varphi = \frac{1}{3} \frac{d\rho}{dt},$$

wo φ die Deviation ebener Kurve sind.

Aus (8) ergibt sich

$$(9) \quad \tan \varphi = \frac{1}{3} \frac{d\rho}{d\sigma},$$

wo $d\sigma$ den Kontingenzwinkel von ξ bedeutet.⁽¹⁾

Wenn $\frac{d\rho}{d\sigma} = \infty$, so folgt $\varphi = \frac{\pi}{2}$ in (9), wenn $\frac{d\rho}{d\sigma} = 0$, so folgt $\varphi = 0$.

Der Winkel σ ist eine Funktion von t , und die Gleichung

$$(10) \quad \frac{d\sigma}{dt} = \frac{1}{\sigma}$$

sagt uns, dass die Krümmung gerade die Ableitung dieser Funktion ist.

Daraus folgt

$$(11) \quad \sigma = \int_0^t \frac{dt}{\rho},$$

wenn der Winkel von dem Kreise in dem Anfangspunkte des Bogens gerechnet wird, vorausgesetzt, dass das Integral einen Sinn hat.

Die Funktion σ ist für die Discussion der ebenen Kurven, die durch ihre natürliche Gleichung gegeben sind.

Wenn σ mit der Annäherung von t an eine bestimmte endliche

(1) MATSUMURA, S.: Über einen affingeometrischen Satz und die Deviation ebener Kurven, Tôhoku Math. Journ. 36 (1933) p. 189.

oder unendliche Grenze unendlich gross wird, so existiert in dem entsprechenden Punkte kein Berührungskreis mehr.

Wir werden immer voraussetzen, dass dies nur in den isolierten Punkten eintritt.

Wir wollen für einen Augenblick den Anfangspunkt des Bogens t nach A verlegen und A' in der genügenden Nähe von A wählen, um auf dem Bogen AA' jeden Punkt mit unbestimmter Tangente auszuschliessen.

Man kann setzen

$$(12) \quad \begin{cases} dt^2 = du^2 + dv^2, & \lim \frac{v}{u} = 0, & \lim \frac{\delta t}{u} = 1, \\ \lim \frac{v}{u^2} = \frac{1}{2} \lim \frac{\tan \sigma}{u} = \frac{1}{\rho}, \end{cases}$$

wenn A' nach A hinrückt.

Daraus ergibt sich :

$$(13) \quad \rho^2 d\sigma^2 = du^2 + dv^2,$$

$$\text{d.h. (13')} \quad \frac{du}{dt} = \cos \sigma, \quad \frac{dv}{dt} = \sin \sigma,$$

$$\text{d.h. (14)} \quad u = \int_0^\sigma \rho \cos \sigma \, d\sigma, \quad v = \int_0^\sigma \rho \sin \sigma \, d\sigma.$$

Wir haben

$$(15) \quad \frac{d\sigma}{dt} = (\dot{\zeta}_t, \dot{\zeta}_t) = -\frac{1}{\rho};$$

man kann also die Krümmung auch betrachten als den Grenzwert des Verhältnisses des Winkels $\delta\sigma$ zu dem Bogen δt .

Offenbar variiert ρ im allgemeinen von einem Kurvenpunkte zu einem andern, d. h. die Krümmung ist eine Funktion des Bogens, und es zeigt sich sehr bald, dass die Kenntnis dieser Funktion genügt, um die Gestalt der Kurve zu bestimmen.

Aus diesem Grunde ist

$$(16) \quad f(t, \sigma) = 0$$

die natürliche Gleichung dieser Kurve.

Nach Berechnung von σ wird die Integration der Formeln (13') als Funktionen von σ geliefert.

Ist $\hat{\xi}(\sigma) ((\hat{\xi}\hat{\xi})=1)$ der Schmiegungskreis einer Kurve $\mathbf{b}=\mathbf{b}(\sigma)$ im R_2 , so lässt sich ein beliebiger Tangentialkreis in $\mathbf{b}(\sigma)$ durch

$$(17) \quad \varphi(\sigma) = \hat{\xi}(\sigma) + F(\sigma)\mathbf{b}(\sigma), \quad ((\varphi\varphi)=1)$$

darstellen.

Die tetrazyklischen Koordinaten $\hat{\xi}(\sigma)$ des Schmiegungskreises sind unter den verschiedenen Tangentialkreisen in $\mathbf{b}(\sigma)$ durch die folgende Bedingung gekennzeichnet:

$$(18) \quad (d\hat{\xi} d\hat{\xi})=0.$$

Durch Spezialisierung der Funktion $F(\sigma)$ wird der Tangentialkreis an ein und derselben Stelle σ individualisiert.⁽¹⁾

Aus (17) ergibt sich

$$-\frac{d\sigma}{dt} = \frac{1}{\rho}$$

Nun betrachten wir

$$(19) \quad \left\{ \begin{aligned} \frac{\varphi(\sigma) - \varphi(\sigma_0)}{F(\sigma) - F(\sigma_0)} &= \frac{\hat{\xi}(\sigma) - \hat{\xi}(\sigma_0) + F(\sigma)\mathbf{b}(\sigma) - F(\sigma_0)\mathbf{b}(\sigma_0)}{F(\sigma) - F(\sigma_0)} \\ &= \frac{\hat{\xi}(\sigma) - \hat{\xi}(\sigma_0) + F(\sigma_0)(\mathbf{b}(\sigma) - \mathbf{b}(\sigma_0))}{F(\sigma) - F(\sigma_0)} + \mathbf{b}(\sigma), \end{aligned} \right.$$

wo bekanntlich

$$F(\sigma) = \frac{1}{\rho(\sigma)} \quad \text{ist.}$$

$$\begin{aligned} \lim_{\sigma \rightarrow \sigma_0} \frac{\hat{\xi}(\sigma) - \hat{\xi}(\sigma_0) + F(\sigma_0)(\mathbf{b}(\sigma) - \mathbf{b}(\sigma_0))}{F(\sigma) - F(\sigma_0)} \\ = \lim \frac{\hat{\xi}'(\sigma) + F(\sigma_0)\mathbf{b}'(\sigma)}{F'} \end{aligned}$$

(1) THOMSEN, G.: op. cit.

$$= -\frac{\frac{s}{\rho(\sigma)} + \frac{s}{\rho(\sigma_0)}}{\rho\left(\xi \frac{d^2 v}{d\sigma^2}\right)},$$

wo

$$\frac{dv}{d\sigma} = s, \quad \frac{d\xi}{d\sigma} = -\frac{s}{\rho}$$

gesetzt sind.⁽¹⁾

So folgt der

Satz :

$$(20) \quad \lim_{\sigma \rightarrow \sigma_0} \frac{\frac{\varphi(\sigma) - \varphi(\sigma_0)}{1} - \frac{1}{\rho(\sigma)}}{\frac{1}{\rho(\sigma_0)}}$$

ist mit

$$(21) \quad v(\sigma) + \left\{ \left(-\frac{s}{\rho(\sigma)} + \frac{s}{\rho(\sigma_0)} \right) / \rho\left(\xi \frac{d^2 v}{d\sigma^2}\right) \right\}$$

gegeben.

$\sigma = \sigma(t)$ besitzt eine inverse Funktion $t = t(\sigma)$, und man erkennt, dass man statt des Parameters σ längs unserem Kurvenbogen die Bogenlänge t als Parameter einführen kann.

Dann wird $\xi = \xi(t(\sigma)) = \bar{\xi}(\sigma)$ eine stetige Funktion, für die auch $\frac{d\bar{\xi}}{dt}$ stetig ist.

Im allgemeinen ist

$$\frac{d\bar{\xi}}{d\sigma} \neq 0,$$

denn es ist ja

$$(22) \quad \frac{d\bar{\xi}}{d\sigma} = -\frac{d\bar{\xi}}{dt} \cdot \frac{dt}{d\sigma} = -\frac{d\bar{\xi}}{dt} \cdot \frac{1}{(\xi, \bar{\xi})}.$$

Wenn $v(\sigma)$ Minimallinien oder isotrope Kurven sind, so folgt

(1) TAKASU, T.: Differentialkugelgeometrie, XI, Japanese Journ. of Math. Vol. X (1933) p. 37.

$$\left(\frac{dv}{d\sigma} \frac{d\tilde{v}}{d\sigma} \right) = 0,$$

$$\rho^2 \left(\frac{d\tilde{\xi}}{d\sigma} \frac{d\tilde{\xi}}{d\sigma} \right) = 0,$$

oder

$$\left(\frac{d\tilde{\xi}}{d\sigma} \frac{d\tilde{\xi}}{d\sigma} \right) = 0, \quad (\rho^2 \neq 0), \quad (\text{Minimalkugelschar!})$$

bei denen sich konsekutive Kreise berühren.

Durch $\frac{d\tilde{\xi}}{d\sigma}$ wird dann der Berührungspunkt von $\tilde{\xi}$ mit $\tilde{\xi} + \frac{d\tilde{\xi}}{d\sigma} dt$ dargestellt. Die Kreise $\tilde{\xi}(\sigma)$ sind dann die Schmiegkreise der Kurve $\tilde{\xi}'$.

Für die zu v parallele Kurve besteht

$$(23) \quad -\frac{dt}{d\sigma} = (\tilde{\xi}, \tilde{\xi}_t)^{-1} = \rho + \text{const.}$$

d. h.

$$(24) \quad -\frac{d}{dt} (\tilde{\xi}_t, \tilde{\xi}_t)^{-1} = -\frac{d\rho}{dt}.$$

Wenden wir uns wieder zur relativen Differentialkugelgeometrie, so ergibt sich aus (5):

$$(25) \quad u = \int I'^{-\frac{1}{3}} dt = \int I'^{\frac{2}{3}} d't, \quad \lambda = \lambda(\omega),$$

wo ω die relative Affinbogenlänge und λ die relative Affinkrümmung ist.⁽¹⁾

Ein Kurvenpunkt, in welchem der R.-Krümmungsradius einen stationären Wert besitzt ($I''=0$ in (5)), sei R.-Scheitel genannt.

Bezeichnet man ferner $\tilde{\xi}$ als den Einheitsvektor der äusseren Normalen von v , so folgt für die Parallelkurve

$$(26) \quad v^* = v + c\tilde{\xi}_\lambda \quad (c = \text{const.}).$$

(1) MATSUMURA, S.: Das Extremalproblem der relativen Affinlänge in der relativen Affingeometrie ebener Kurven, Tôhoku Math. Journ. Vol. 33 (1931) p. 232.

Sei p der Abstand ihrer Tangente von 0, dann ordnen wir je zwei Punkte von b und c einander zu, für welche die Vektoren ξ miteinander übereinstimmen, und bezeichnen die Grösse

$$(27) \quad r(\xi) = \frac{[b \xi]}{[u \xi]} = \frac{p(\xi)}{q(\xi)}$$

als den R.-Abstand der b -Tangente (ξ) von 0, natürlich in Bezug auf c , wo $[a, b] = a_1 b_2 - a_2 b_1$ ist

Der R.-Bogen t von b wird bestimmt durch

$$(28) \quad \sqrt{b}(t) = \frac{1}{[u \xi]} = \frac{1}{q},$$

wo ich q als den Abstand der Tangente zu c von 0 bezeichne und auch mit φ den Winkel, den die Tangente mit einer festen Richtung bildet.

Für einen beliebigen Parameter τ aus (28) folgt

$$(29) \quad \begin{cases} t = \int [u \xi] \sqrt{b} d\tau \\ = \int q \rho(b) d\varphi = \int q dt(b). \end{cases}$$

Ihm entspricht als Eichbogen

$$(30) \quad \begin{cases} t' = \int [u \xi] \sqrt{u} d\tau = \int q \rho(c) d\varphi \\ = \int \frac{q d\varphi}{(\xi_i, \xi_i)} = \int q dt(c), \end{cases}$$

woraus (5) hervorgeht.

Für R.-Kreise ist Γ konstant.

Ist umgekehrt Γ konstant, so schliesst man aus (5), dass b dem c ähnlich und ähnlich gelegen ist, wo wir b dann einen R.-Kreis in allgemeiner Lage nennen wollen.

Für den gewöhnlichen Flächeninhalt $I(b)$ erhält man

$$(31) \quad 2I(b) = \oint r dt = \oint r \Gamma d't = \oint \frac{r}{(\xi_i, \xi_i)} d't,$$

während der Eichumfang

$$(32) \quad \Sigma = \oint d't = 2I(e)$$

wird.

Für den R.-Umfang S von \mathfrak{b} aber gilt

$$(33) \quad S = \oint dt = \oint \Gamma d't = \oint \frac{d't}{(\xi, \xi_i)} = \oint r d't;$$

denn es ist

$$(34) \quad \left\{ \begin{aligned} S &= \oint \frac{q}{(\xi, \xi_i)} d\varphi = \oint \rho(p + q_{rr}) d\varphi = \oint (pq - p_r q_r) d\varphi \\ &= \oint p(q + q_{rr}) d\varphi = \oint pq = \oint pq(e) d\varphi = \oint \frac{p}{(\xi, \xi_i)} d\varphi \end{aligned} \right.$$

S bleibt bei Translation erhalten.

Ferner gehen wir zur Affingeometrie in R.-Geometrie über.

Ist \bar{u} die Affinbogenlänge von \mathfrak{b} und \bar{v} die von e , so ist bekanntlich

$$(35) \quad \left\{ \begin{aligned} \frac{d\bar{u}}{d\varphi} - \rho(\mathfrak{b})^{\frac{2}{3}} &= (\xi, \xi_i)^{-\frac{2}{3}}, \\ \frac{d\bar{v}}{d\varphi} &= \rho(e)^{\frac{2}{3}} = (\xi', \xi'_i)^{-\frac{2}{3}}, \\ \frac{d\bar{u}}{d\bar{v}} &= \Gamma^{\frac{2}{3}} = (\xi', \xi'_i)^{\frac{2}{3}} \cdot (\xi, \xi_i)^{\frac{2}{3}}. \end{aligned} \right.$$

Wählen wir als Eichkurve e das Affinkrümmungsbild r_{uu}^- von \mathfrak{b} , das vielleicht keine Eilinie ist, so ist

$$(36) \quad q = [e \xi] = \rho(e)^{-\frac{1}{3}} = (\xi, \xi_i)^{-\frac{1}{3}},$$

und es wird t bis auf eine zu vernachlässigende additive Konstante selbst zum Affinbogen

$$(37) \quad \left\{ \begin{aligned} t &= \int q \rho(\mathfrak{b}) d\varphi = \int \rho(\mathfrak{b})^{\frac{2}{3}} d\varphi \\ &= \int (\xi, \xi_i)^{-\frac{2}{3}} d\varphi = \int d\bar{u}. \end{aligned} \right.$$

Ferner wird, wenn K die Affinkrümmung von \mathfrak{v} bedeutet,

$$(38) \quad \mathfrak{v}' = \Gamma \mathfrak{e}' = -\Gamma \mathfrak{v}''' = \Gamma K \mathfrak{v}' = \frac{(\xi_i' \xi_i') K \mathfrak{v}'}{(\xi_i, \xi_i)};$$

also ist Γ gerade der Affinkrümmungsradius von \mathfrak{v} .

Schliesslich wird die R.-Entfernung r zur Affinentfernung ¹⁾

$$r = p/q = o(\mathfrak{v})^{\frac{1}{3}} (\mathfrak{v} \xi) = (\mathfrak{v} \mathfrak{x}).$$

Die Affingeometrie einer Kurve ist also ihre R.-Geometrie bezüglich ihres Affinkrümmungsbildes.

Das Affinkrümmungsbild ist auf jeden Fall geschlossen.

Es ist zugleich eine Eilinie, wenn \mathfrak{v} elliptisch gekrümmt, d. h. wenn stets

$$(39) \quad \Gamma' > 0$$

ist; dann gelten alle Formeln also auch hier.

Danach ist ein Beweis mancher bekannten Sätze der affinen Kurventheorie leicht geworden, z. B. es folgt:

$$(40) \quad S\Gamma_{\text{Min}} \leq 2I(\mathfrak{v}) \leq S\Gamma_{\text{Max}}.$$

Unter allen Eilinen gegebenen Inhalts haben die Ellipsen das grösste Integral der Affinkrümmung

$$(41) \quad \Sigma = \oint \frac{dt}{\Gamma}.$$

Ist für eine elliptisch gekrümmte Eilinie

$$(42) \quad \Gamma = \text{const. oder } r = \text{const.},$$

so ist eine Ellipse da,

Mit gleicher Methode kann man relativdifferentialkugelgeometrische Erweiterung der Affingeometrie untersuchen. ⁽²⁾

Es sei $\bar{u}(\phi)$ der Affinbogen von \mathfrak{v} , $\bar{v}(\phi)$ der von \mathfrak{e} ; ferner sei $f = \rho(\mathfrak{e})^{\frac{1}{3}} \xi$, $\mathfrak{x} = \rho(\mathfrak{v})^{\frac{1}{3}} \xi$ und $P = (\mathfrak{v} \mathfrak{x})$ die negative Affinentfernung der Kurve \mathfrak{v} von 0 entsprechend $Q = (\mathfrak{e} f)$. Dann definieren wir

(1) SÜSS, W.: op. cit.

(2) MATSUMURA, S.: op. cit.

$$(43) \quad \left\{ \begin{array}{l} u = \int (ef) d\bar{u} = \int Q d\bar{u} = \int \rho(e)^{\frac{1}{3}} q \rho(v)^{\frac{2}{3}} d\varphi \\ \quad = \int \Gamma^{-\frac{1}{3}} dt = \int \Gamma^{\frac{2}{3}} d't \end{array} \right.$$

als R.-Affinbogen von ξ , während die Grösse

$$(44) \quad v = \int (ef) d\bar{v} = \int Q d\bar{v} = \int \rho(e) q d\varphi = 't$$

mit dem Eichbogen übereinstimmt.

· Die Formel für den R.-Affinumfang

$$(45) \quad U = \oint \Gamma^{\frac{2}{3}} d't = \oint \Gamma^{-\frac{1}{3}} dt$$

entspricht dabei ganz der für den gewöhnlichen Affinumfang

$$(46) \quad U = \oint d(v)^{\frac{2}{3}} d\varphi = \oint \rho(v)^{-\frac{1}{3}} dt(v).$$

Nach der Hölderschen Ungleichung wird ferner

$$(47) \quad U \leq \int d't \left(\oint \Gamma d't \right)^{\frac{1}{2}} = \sum S^2,$$

worin das Gleichheitszeichen nur für

$$(48) \quad \Gamma = \text{const.},$$

d. h. nur für R.-Kreise gilt.

Vielmehr kann man ausser U die Grösse

$$(49) \quad W = \oint \frac{(v\bar{x})}{(ef)} d't = \oint v d't = \oint (v\bar{x}) d\bar{v} \\ = \oint P d\bar{v} \left(E = \frac{P}{Q} = r \Gamma^{\frac{1}{3}} \right),$$

eingeführen, für welche die Abschätzung

$$(50) \quad W \leq \frac{S^4}{\sum}$$

gilt.

Auch ist noch

$$(51) \quad UW \leq S^2,$$

worin das Gleichheitszeichen wieder für R.-Kreise kennzeichnend ist.

Setzen wir noch

$$(52) \quad \mathfrak{z} = \frac{r^{\frac{1}{3}}}{q} \xi$$

als Gegenstück zu

$$(53) \quad \mathfrak{x} = \rho(v)^{\frac{1}{3}} \xi$$

in der gewöhnlichen Affingeometrie, so ist $E=(v\mathfrak{z})$ die negative R.-Affinentfernung von v von 0 und es folgt

$$(54) \quad 2I(v) = \oint E \, du.$$

Ist e die Einheitsellipse, d. h. $(ef)=Q=1$, so entstehen die Formeln der Affingeometrie; U wird zum gewöhnlichen Affinumfang, $\Sigma=2\pi$, und (45) lässt sich zur isoperimetrischen Ungleichung der Affingeometrie erweitern:

$$(55) \quad U^3 \leq 2\Sigma I^2(v)$$

In ihr gilt das Gleichheitszeichen, falls v eine Ellipse, hier also mit e affinverwandt ist.

Es kann vermutet werden, dass (55) ganz allgemein gilt und das Gleichheitszeichen für Affinverwandtschaft zwischen v und e kennzeichnend bleibt.

Ausserdem ergibt sich dann aus (50) und (51):

$$(56) \quad W^3 \leq 4\Sigma I^2(v), \quad UW \leq 2\Sigma I(v)$$

mit derselben Bedeutung des Gleichheitszeichens.

Falls sich (55) und (56) beweisen lassen, so folgt es aus $E=\text{const.}$, dass v mit e affinverwandt und zugleich ein R.-Affinkreis ist; denn dann ist

$$(57) \quad 2I=EU, \quad W=EW, \quad UW=2\Sigma I.$$

Als Vektor der R.-Affinnormale führen wir ein:

$$(58) \quad \mathfrak{v} = (ef)^s \frac{d^2 \mathfrak{v}}{du^2} = Q^s \mathfrak{v}''.$$

Dann gilt wie in der Affingeometrie

$$(59) \quad (\mathfrak{v}\mathfrak{z}) = (\mathfrak{v}', \mathfrak{v}) = 1; \quad (\mathfrak{v}\mathfrak{z}') = (\mathfrak{v}'\mathfrak{z}) = (\mathfrak{v}', \mathfrak{v}') = 0$$

und die Grösse λ in

$$(60) \quad \mathfrak{v}' = \lambda \mathfrak{v}'$$

ist als R.-Affinkrümmungsradius zu betrachten.

$\lambda = \lambda(u)$ ist die natürliche Gleichung der Kurve \mathfrak{v} in Bezug auf e . Bezogen auf das R.-Affinkrümmungsbild \mathfrak{v} als Eichkurve wird jetzt übrigens wegen $\mathfrak{v}\bar{u}^2 = \rho(\mathfrak{v})^{\frac{2}{3}}$:

$$(61) \quad u = \int (\mathfrak{v}\hat{z}) dt(\mathfrak{v})$$

der R.-Bogen der elementaren R.-Geometrie, $\lambda = \frac{dt(\mathfrak{v})}{dt(\mathfrak{v})}$ der R.-Krümmungsradius, für den ein Vierscheitelsatz gilt, und

$$(62) \quad \frac{(\mathfrak{v}\hat{z})}{(\mathfrak{v}\hat{z})} = \frac{\rho I^{\frac{1}{3}}}{q} = I^{\frac{1}{3}} = E$$

der negative R.-Abstand.

Die Durchführung der oben erörterten Geometrie ist insbesondere auch für die Fläche möglich.

Hier betrachten wir zwei Systeme von den Kreisen im R_2 und setzen

$$(63) \quad \hat{z} = \hat{z}(\sigma), \quad \mathfrak{x} = \mathfrak{x}(\sigma)$$

und machen zwei Hüllkurven $\mathfrak{v} = \mathfrak{v}(\sigma)$ und $\mathfrak{w} = \mathfrak{w}(\sigma)$ geltend, dann ergibt sich:

$$(64) \quad \cos \theta = - \frac{\mathfrak{v}_\sigma \mathfrak{w}_\sigma}{\sqrt{\mathfrak{v}_\sigma^2 \cdot \mathfrak{w}_\sigma^2}} = - \frac{\hat{z}_\sigma \mathfrak{x}_\sigma}{\sqrt{\hat{z}_\sigma^2 \mathfrak{x}_\sigma^2}},$$

wo θ der Winkel zwischen zwei Richtungen von Kurven im Schnittpunkt ist.

Affinlänge s ist mit

$$(65) \quad s = \int_{t_0}^t \left[-\frac{dv}{dt}, \frac{d^2v}{dt^2} \right]^{\frac{1}{3}} dt$$

gegeben, wo

$$(66) \quad v_0 = -c \xi_0, \quad \frac{d\sigma}{dt} = -\frac{1}{\rho}.$$

Setzen wir auf jede Tangente je einen Punkt η bzw. $\bar{\eta}$, so folgt

$$(67) \quad \eta = v + l v_0, \quad \bar{\eta} = \bar{v} + \bar{l} \bar{v}_0,$$

wo l, \bar{l} die Länge zwischen v und η bzw. \bar{v} und $\bar{\eta}$ bedeuten, so ergibt sich:

$$(68) \quad \begin{cases} \xi_{\sigma\sigma} = -\xi + c(\eta - l v_0) + c(\bar{\eta} - \bar{l} \bar{v}_0), \\ \eta = v - cl \xi_0, \\ \bar{\eta} = \bar{v} - \bar{c} \bar{l} \xi_0 \end{cases}$$

(68) ist die Bedingung dafür, dass zwischen zwei Örtern v und \bar{v} bestehen müssen.

Wenn v eine Eilinie bilden, so folgt

$$(69) \quad \begin{cases} [\dot{v}, \ddot{v}] \geq 0 \\ \text{oder } [\dot{v}, \ddot{v}] \leq 0. \end{cases}$$

Nun bestehen

$$(70) \quad 8\pi^2 F - S' \geq 0,$$

wo

$$(71) \quad S = \oint [\dot{v}, \ddot{v}]^{\frac{1}{3}} dt,$$

F ist hier der Flächeninhalt einer geschlossenen v -Kurve.

Affinkrümmung ist mit

$$(72) \quad k = [\dot{v}, \ddot{v}]$$

gegeben.

Der Affinmittelpunkt ist mit

$$(73) \quad \eta = b + \frac{1}{k} \ddot{b}$$

gegeben, wo

$$(74) \quad b_\sigma = -\hat{\xi}_\sigma, \quad \bar{b}_\sigma = -\bar{c} \bar{\xi}_\sigma$$

sind.

(2) Grundlage der konformen Flächentheorie

Betrachten⁽¹⁾ wir eine quadratische Form

$$(1) \quad G_{ik} du^i du^k, \quad (i, k=1, 2),$$

wo

$$(2) \quad G_{ik} \equiv (\theta_i, \theta_k), \quad u^1 = t, \quad u^2 = \tau$$

bestehen.

G_{ik} ist ein kovarianter symmetrischer Tensor zweiter Stufe ($G_{ik} = G_{ki}$), dessen Komponenten gegebene Funktionen der Koordinaten u^i des Kreises sind.

Der Abstand ds zweier Nachbarpunkte wird durch die Formel

$$(3) \quad ds^2 = G_{ik} du^i du^k$$

gegeben.

Die Nulllinien unserer Form sind die Minimallinien.

Wir bezeichnen mit G die Determinante von G_{ik} , die wir als $\neq 0$ voraussetzen, und führen den schiefsymmetrischen kovarianten Tensor e^{ik} mit den Komponenten

$$(4) \quad e^{11} = 0, \quad e^{12} = \frac{1}{\sqrt{G}}, \quad e^{21} = -\frac{1}{\sqrt{G}}, \quad e^{22} = 0$$

ein.

(1) NAKAJIMA, S.: Kugelgeometrie von Mobius, Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ. Vol. II (1929) p. 36.

Bezeichnen wir mit θ_{ik} die zweiten kovarianten Ableitungen θ in Bezug auf die Form G_{ik} , so ist

$$(5) \quad \frac{1}{4} e^{rs} e^{pq} | \theta, \theta_r, \theta_s, \theta_{ip}, \theta_{kd} | du^i du^k = p_{ik} du^i du^k$$

gleichfalls eine halbinvariante, parameterinvariante Form.

Ihre Nulllinien sind wie wohl bekannt die *Krümmungslinien*.

So ergibt sich:

$$(6) \quad (\theta_i \theta_j) p_{11} - 2(\theta_i \theta_j) p_{12} + (\theta_i \theta_j) p_{22} = 0.$$

Wenn die Krümmungslinien Parameterkurven sind, so folgt:

$$p_{11} = p_{22} = 0.$$

Wir können ihre Differentialgleichung in der Form schreiben:

$$(7) \quad \left\{ \begin{array}{l} | \theta, \theta_1, \theta_2, d\theta_1, d\theta_2 | = 0, \\ \text{d. h. } | \cos \tau \mathfrak{C}(t) + \sin \tau \mathfrak{D}(t) + i \eta(t) \\ \quad - A \cos \tau \mathfrak{A} - B \sin \tau \mathfrak{B} - (S \sin \tau + i T) \mathfrak{C} + (S \cos \tau - i V) \mathfrak{S} \\ \quad \quad \quad + (T \cos \tau + V \sin \tau) \eta, \\ \quad - \sin \tau \mathfrak{C} + \cos \tau \mathfrak{D}, \\ \quad d(-A \cos \tau \mathfrak{A} - B \sin \tau \mathfrak{B} - (S \sin \tau + i T) \mathfrak{C} + (S \cos \tau - i V) \mathfrak{S} \\ \quad \quad \quad + (T \cos \tau + V \sin \tau) \eta), \\ \quad d(-\sin \tau \mathfrak{C} + \cos \tau \mathfrak{D}) | = 0, \end{array} \right.$$

und man kann sie nach meiner Arbeit⁽¹⁾ ausrechnen.

Normieren wir den Flächenpunkt θ durch

$$\tilde{\theta} = \theta \sqrt{-\frac{p}{g}},$$

so haben wir

(1) THOMSEN, G.: op. cit.

$$(8) \quad \left\{ \begin{array}{l} G_{ik} = -g_{ik} \frac{p}{g} = (\theta_i, \theta_k), \\ P_{ik} = p_{ik} \sqrt{-\bar{p}} = \frac{1}{2} E^{rs} E^{pq} | \tilde{\theta}, \tilde{\theta}_r, \tilde{\theta}_s, \tilde{\theta}_{ip}, \tilde{\theta}_{kq} |, \\ \left[E^{11} = 0, E^{12} = \frac{1}{\sqrt{G}}, E^{21} = -\frac{1}{\sqrt{G}}, E^{22} = 0, G = G_{ik} | \right] \end{array} \right.$$

wo $p = p_{ik} |$ ist.

Setzen wir

$$(9) \quad G^{ik} = E^{ir} E^{ks} G_{rs},$$

so ist mit

$$(10) \quad \gamma = \frac{1}{2} E^{rs} G^{ik} | \tilde{\theta}, \tilde{\theta}_r, \tilde{\theta}_s, \tilde{\theta}_{ik} |,$$

die *Zentralkugel* der Fläche $\theta(t, \tau)$ im Punkte t, τ bestimmt.

Wenn

$$G^{11} = G^{22} = 0,$$

so folgt

$$(11) \quad \gamma = \rho | | \theta, \theta_u, \theta_v, \theta_{uv} | |.$$

Betrachten wir eine dritte invariante Differentialform zweiter Ordnung

$$(12) \quad (\gamma d^2 \tilde{\theta}) = \frac{1}{4} E^{rs} G^{pq} | \tilde{\theta}, \tilde{\theta}_r, \tilde{\theta}_s, \tilde{\theta}_{pq}, \tilde{\theta}_{ik} | du^i du^k \\ = C_{ik} du^i du^k,$$

so bezeichnet (12) die *Schnitttangentenkurven* der Fläche θ .

Zwischen den vier Grundtensoren G_{ik} , P_{ik} , C_{ik} , E_{ik} bestehen

$$(13) \quad \left\{ \begin{array}{l} \text{(a) } \frac{1}{2} G^{ik} G_{ik} = 1, \quad \text{(b) } \frac{1}{2} P^{ik} P_{ik} = 1, \\ \text{(c) } \frac{1}{2} C^{ik} C_{ik} = 1, \quad \text{(d) } \frac{1}{2} E^{ik} E_{ik} = 1, \end{array} \right.$$

$$(14) \quad \left\{ \begin{array}{ll} (a) & G^{ik} P_{ik}=0, \quad (b) \quad G^{ik} C_{ik}=0, \\ (c) & P^{ik} C_{ik}=0, \quad (d) \quad E^{ik} G_{ik}=0, \\ (e) & E^{ik} P_{ik}=0, \quad (f) \quad E^{ik} C_{ik}=0, \end{array} \right.$$

$$(15) \quad (a) \quad P_{ik}=E_{ir} C_{rk}, \quad (b) \quad C_{ik}=E_{ir} P_{rk},$$

$$(16) \quad (a) \quad G_{ik}=C_{ir} C_{rk}; \quad (b) \quad G_{ik}=P_{ir} P_{rk},$$

$$(17) \quad (a) \quad E^{ir} E^{ks} P_{rs} = -P^{ik}, \quad (b) \quad E^{ir} E^{ks} C_{rs} = -C^{ik},$$

$$(18) \quad P_{il} C_k^l = C_{kl} P_i^l = E_{ik},$$

$$(19) \quad E^{is} E_{ks} = G_k^i = \begin{cases} 1 & i=k, \\ 0 & i \neq k, \end{cases}$$

$$(20) \quad G = -P = -C = E,$$

$$(21) \quad G^{ik} P_{ik}=0,$$

dann entstehen die folgenden *Ableitungsgleichungen*

$$(22) \quad \left\{ \begin{array}{l} \theta_{ik} = A_{ik} \theta + C_{ik} \eta + G_{ik} \xi, \\ \eta_r = M_r \theta - C_r^i \theta_i, \\ \xi_r = A_r^i \theta_i + M_r \eta, \end{array} \right.$$

wo

$$(23) \quad \left\{ \begin{array}{l} (\theta\theta)=0, (\theta\theta_i)=0, (\theta\eta)=0, (\theta\xi)=-1, (\theta\theta_{ik})=-G_{ik}, \\ (\theta_i\theta_k)=G_{ik}, (\theta_i\eta)=0, (\theta_i\xi)=0, (\theta_i\theta_{kl})=0, \\ (\eta\eta)=1, (\eta\xi)=0, (\eta\theta_{ik})=C_{ik}, \\ (\xi\xi)=0, (\xi\theta_{ik})=-A_{ik}, \\ (\tilde{\theta}\eta_r)=0, (\tilde{\theta}_i\eta_r)=-C_{ir}, (\eta\eta_r)=0, (\xi\eta_r)=-M_r, \\ (\tilde{\theta}\xi_r)=0, (\theta_i\xi_r)=A_{ir}, (\eta\xi_r)=M_r, (\xi\xi_r)=0. \end{array} \right.$$

Die Integrabilitätsbedingungen sind :

$$(24) \quad \left\{ \begin{array}{l} \tilde{\theta}_{ikr} - \tilde{\theta}_{irk} = R_{imrk} G^m \tilde{\theta}_i, \\ \eta_{rs} - \eta_{sr} = 0, \\ \xi_{rs} - \xi_{sr} = 0 \end{array} \right.$$

(Jeder neu angehängte Index bedeutet kovariante Ableitung.)

$$(25) \quad R_{imrk} = \frac{\partial \Gamma_{ir,m}}{\partial u^k} - \frac{\partial \Gamma_{ikm}}{\partial u^r} + \Gamma_{iks} \Gamma_{mr}^s - \Gamma_{irs} \Gamma_{mk}^s$$

ist der *Riemannsche Krümmungstensor* der Form G_{ik} .

Zwischen drei Invarianten

$$(26) \quad \begin{cases} J = G^{ik} M_i M_k, \\ S = P^{ik} M_i M_k, \\ T = C^{ik} M_i M_k \end{cases}$$

besteht

$$(27) \quad J^2 - S^2 - T^2 = 0.$$

Wollen wir zwei Wurzeln von

$$(28) \quad p_{11} \left(\frac{du_1}{du_2} \right)^2 + 2p_{12} \left(\frac{du_1}{du_2} \right) + p_{22} = 0$$

mit k_1, k_2 bezeichnen, so folgt

$$(29) \quad \begin{cases} k_1 = \frac{-p_{12} + \sqrt{p_{12}^2 - p_{11} p_{22}}}{p_{11}}, \\ k_2 = \frac{-p_{12} - \sqrt{p_{12}^2 - p_{11} p_{22}}}{p_{11}}. \end{cases}$$

Daraus ergibt sich

$$(30) \quad \begin{cases} (\theta_i \theta_i) + (\theta_i \theta_\tau) (k_1 + k_2) + (\theta_\tau \theta_\tau) k_1 k_2 = 0, \\ L + M(k_1 + k_2) + N k_1 k_2 = 0, \\ L p_2 - 2 p_{12} M + N p_1 = 0, \end{cases}$$

wo L, M, N zweiter Fundamentalgrößen der Fläche $\theta(t, \tau)$ sind.

Man kann den Radius

$$(31) \quad \frac{1}{H} = \frac{1}{2} \left(-\frac{1}{R_1} + \frac{1}{R_2} \right)$$

von der Zentralkugel berechnen, wo R_i die Hauptkrümmungsradien sind.

Nun wollen wir

$$(\theta_t \theta_t), (\theta_t \theta_\tau), (\theta_\tau \theta_\tau)$$

die modifizierten ersten Fundamentalgrößen der Fläche nennen, wo

$$(32) \quad (\theta_t \theta_t) = E, \quad (\theta_t \theta_\tau) = F, \quad (\theta_\tau \theta_\tau) = G$$

bestehen, dann kann man⁽¹⁾ aus

$$(33) \quad \begin{cases} (\theta_t \theta_t) q_{12} - (\theta_t \theta_\tau) q_{11} = p_{11}, \\ (\theta_\tau \theta_\tau) q_{11} - (\theta_t \theta_t) q_{22} = -2p_{11}, \\ (\theta_t \theta_t) q_{22} - 2(\theta_t \theta_\tau) q_{12} + (\theta_\tau \theta_\tau) q_{11} = \sqrt{(\theta_t \theta_t)(\theta_\tau \theta_\tau) - (\theta_t \theta_\tau)^2} H, \end{cases}$$

rechnen:⁽¹⁾

$$q_{11}, \quad q_{12}, \quad q_{22}$$

wo

$$(\theta_t \theta_t), (\theta_t \theta_\tau), (\theta_\tau \theta_\tau); \quad q_{11}, \quad q_{12}, \quad q_{22},$$

die modifizierten ersten Fundamentalgrößen bzw. zweiten Fundamentalgrößen der Fläche $\theta(t, \tau)$ sind, wo $t \equiv u_1$, $\tau \equiv u_2$ sind.

Also ergibt sich

(A) für Differentialgleichung der Haupttangenten-kurve :

$$(34) \quad q_{11} du^2 + 2q_{12} du dv + q_{22} dv^2 = 0.$$

(B) für modifiziertes GAUSZsches Krümmungsmasz :

$$(35) \quad k = (q_{11} q_{22} - q_{12}^2) / \{(\theta_t \theta_t)(\theta_\tau \theta_\tau) - (\theta_t \theta_\tau)^2\}, \text{ u. s. w.}$$

Ein isogonales Strahlennetz lässt sich stets durch eine konforme Abbildung der Fläche in das System aller Geraden der Ebene überführen.

Die Bedingung für ein Strahlennetz kann hingegen so formuliert werden :

⁽¹⁾ THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ. IV (1915) S. 138.

$$(36) \quad \frac{\partial}{\partial t} \left(\frac{(\theta_\tau \theta_\tau)U - (\theta_t \theta_\tau)V}{\sqrt{(\theta_t \theta_t)(\theta_\tau \theta_\tau) - (\theta_t \theta_\tau)^2}} \right) = \frac{\partial}{\partial \tau} \left(\frac{(\theta_t \theta_t)U - (\theta_t \theta_\tau)V}{\sqrt{(\theta_t \theta_t)(\theta_\tau \theta_\tau) - (\theta_t \theta_\tau)^2}} \right),$$

Wenn die Fläche eine Kreisfläche ist, wo U und V zwei Ortsfunktionen auf der Kreisfläche sind.⁽¹⁾

Für

$$q_{11}, \quad q_{12}, \quad q_{22}$$

besteht natürlich folgender Satz:

Versteht man unter $\frac{1}{\rho}$, $\frac{1}{\rho_1}$ die Normalkrümmungen, welche

durch die Ausdrücke

$$(37) \quad \begin{cases} \frac{1}{\rho} = \frac{q_{11} d\bar{t}^2 + 2q_{12} dt d\bar{\tau} + q_{22} d\bar{\tau}^2}{(\theta_t \theta_t) d\bar{t}^2 + 2(\theta_t \theta_\tau) dt d\bar{\tau} + (\theta_\tau \theta_\tau) d\bar{\tau}^2}, \\ \frac{1}{\rho_1} = \frac{\bar{q}_{11} d\bar{t}^2 + 2\bar{q}_{12} dt d\bar{\tau} + \bar{q}_{22} d\bar{\tau}^2}{(\theta_t \theta_t) d\bar{t}^2 + 2(\theta_t \theta_\tau) dt d\bar{\tau} + (\theta_\tau \theta_\tau) d\bar{\tau}^2} \end{cases}$$

gegeben sind, so zeigt sich, dass durch jeden Punkt Richtungen gehen, für welche

$$\frac{1}{\rho^2} = \frac{1}{\rho_1^2}$$

wird, bestimmt durch beide Gleichungen

$$(38) \quad \begin{cases} (q_{11} - \bar{q}_{11}) d\bar{t}^2 + 2(q_{12} - \bar{q}_{12}) dt d\bar{\tau} + (q_{22} - \bar{q}_{22}) d\bar{\tau}^2 = 0, \\ (q_{11} + \bar{q}_{11}) d\bar{t}^2 + 2(q_{12} + \bar{q}_{12}) dt d\bar{\tau} + (q_{22} + \bar{q}_{22}) d\bar{\tau}^2 = 0, \end{cases}$$

für welche also die Quadrate der modifizierten Krümmung miteinander gleich sind.

Setzen wir

$$(39) \quad \begin{cases} f = (\theta_t \theta_t) d\bar{t}^2 + 2(\theta_t \theta_\tau) dt d\bar{\tau} + (\theta_\tau \theta_\tau) d\bar{\tau}^2 \\ \varphi = q_{11} d\bar{t}^2 + 2q_{12} dt d\bar{\tau} + q_{22} d\bar{\tau}^2, \end{cases}$$

so ist

$$(40) \quad J(f, \varphi) = 0$$

(1) RADON, J.: Über konforme Geometrie, V, Abh. aus dem Math. Seminar der Hamb. Univ. IV (1926) S. 318.

$$\text{d. h.} \quad p_{11}dt^2 + 2p_{12}dt d\tau + p_{22}d\tau^2 = 0,$$

die Differentialgleichung von Krümmungslinien.⁽¹⁾

Nach OGURA⁽²⁾ ist

$$(41) \quad J(f, J(f, \varphi)) = 0$$

die Differentialgleichung der Schnitttangentenkurve, die den Winkel der Krümmungslinien halbiert.

Schon hat Prof. HAYASHI Torsionschelinien genannt.

Man kann die Gleichung der Torsionschelinien etwas anders ausrechnen :

$$J(f, J(f, \varphi)) = 0.$$

$J(\varphi, J(f, \varphi)) = 0$ sind die Gleichung der charakteristischen Linien.

Nach GAUß⁽³⁾ kann man wissen, dass die notwendige und hinreichende Bedingung dafür, dass beide Flächen aufeinander abwickelbar sind, d.h.

$$\frac{(\theta_i \theta_i)}{(\theta_i \theta_i)} = \frac{(\theta_i \theta_\tau)}{(\theta_i \theta_\tau)} = \frac{(\theta_\tau \theta_\tau)}{(\theta_\tau \theta_\tau)}$$

bestehen, die ist :

$$J_1(K, J_1(K)) = \bar{J}_1(\bar{K}, \bar{J}_1(\bar{K})), \quad J_1(J_1(K)) = \bar{J}_1(\bar{J}_1(\bar{K})),$$

wobei

$$ds^2 = \frac{1}{\lambda} [(\theta_i \theta_i)dt^2 + 2(\theta_i \theta_\tau)dt d\tau + (\theta_\tau \theta_\tau)d\tau^2],$$

$$d\bar{s}^2 = \frac{1}{\bar{\lambda}} [(\bar{\theta}_i \bar{\theta}_i)d\bar{t}^2 + 2(\bar{\theta}_i \bar{\theta}_\tau)d\bar{t} d\bar{\tau} + (\bar{\theta}_\tau \bar{\theta}_\tau)d\bar{\tau}^2]$$

die Bogenelemente der beiden Flächen; J_1 BELTRAMIS erster Differentialparameter und $\frac{\dot{p}_{11}}{\dot{p}_{22}} = K$ bzw. $\frac{\dot{p}_{11}}{\dot{p}_{22}} = K$ GAUßsche Totalkrümmungsmasse der beiden Flächen sind.

(1) Vergl. (28).

(2) OGURA, K.: On the theory of STÄCKEL Curvature, Tôhoku Math. Journ. Vol. 16 (1919) p. 270.

(3) Vergl. GAUSSS Werke.

Wenn

$$\begin{aligned} \Delta_1 K(u, v) &= f(K(u, v)), \\ \bar{\Delta}_1 \bar{K}(\bar{u}, \bar{v}) &= f(\bar{K}(\bar{u}, \bar{v})), \\ J_2(K(u, v)) &= \chi(K(u, v)), \\ \bar{J}_2(\bar{K}(\bar{u}, \bar{v})) &= \chi(\bar{K}(\bar{u}, \bar{v})) \end{aligned}$$

bestehen, dann sind die Flächen auf Rotationsflächen abwickelbar, wo

$$u \equiv t, \quad v \equiv \tau;$$

J_2 BELTRAMIS zweiter Differentialparameter ist.

Die Formel für den Krümmungshalbmesser eines Normalschnitts liefert die Differentialgleichung der charakteristischen Linien in der Gestalt:¹⁾

$$\begin{aligned} &\{-q_{11}p_{12} + 2q_{12}p_{11}\} du^2 \\ &+ \{-q_{12}p_{12} + 2q_{22}p_{11}\} du dv \\ &- \{-q_{22}p_{12} + 2q_{12}p_{22}\} dv^2 = 0. \end{aligned}$$

Betrachten wir eine Kurve auf unserer Fläche $\theta(t, \tau)$ und setzen etwa

$$\begin{aligned} t &= f(x), \\ \tau &= \phi(x), \end{aligned}$$

so folgt

$$\theta(t, \tau) = \theta(f(x), \phi(x)) = \bar{\theta}(x),$$

wo x ein Parameter ist.

Die Bedingung dafür, dass unsere Kurve *Minimallinie* oder *isotrope Kurve* ist, ist mit

$$\left(\frac{d\bar{\theta}}{dx} \quad \frac{d\bar{\theta}}{dx} \right) = 0$$

(1) Vergl. Lilienthal, R.: Zur Theorie der aquidistanten Kurven auf einer Fläche, Math. Ann. Bd. 62, S. 540.

gegeben.

N. B. Es seien Kugelkongruenz

$$(1) \quad \varphi = \varphi(u^1, u^2), \quad (\varphi\varphi) = 1$$

und deren beide Envelopenmäntel

$$(2) \quad \begin{cases} \xi = \xi(u^1, u^2), & (\xi\xi = 0) \\ \bar{\xi} = \bar{\xi}(u^1, u^2), & (\bar{\xi}\bar{\xi} = 0) \end{cases}$$

gegeben, dann entstehen die folgenden Ableitungsgleichungen⁽¹⁾

$$(3) \quad \begin{cases} \varphi_{hk} = -g_{hk}\varphi + D_{hk}\xi + D_{hk}\bar{\xi}, \\ \xi_h = -D'_h\varphi_s + N_h\xi, \\ \bar{\xi}_h = -\bar{D}'_h\varphi_s + N_h\bar{\xi}. \end{cases}$$

Aus (3) ergibt sich

$$\eta_s = B_s^t \{-D'_t\varphi_s - N_t\xi\},$$

wo η den Affinnormalvektor bedeutet.⁽²⁾

Ferner ergibt sich

$$(4) \quad \begin{cases} \mathfrak{X} = \frac{\xi_u \times \xi_v}{F} \\ = \frac{1}{F} \{-D'_u\varphi_s - N_u\xi\} \times \{-D'_v\varphi_s - N_v\xi\}, \end{cases}$$

wo \mathfrak{X} ein kontravarianter Vektor ist, dessen Komponenten die normierten Stellungsparameter der Tangentenebene sind.

Aus

$$(5) \quad \begin{cases} \xi_{ik} = A'_{ik}\xi_i + G_{ik}\xi, \\ \xi = \frac{\xi_1 \times \xi_2}{\bar{G}^{\frac{1}{2}}}, \end{cases}$$

ergeben sich

- (1) TAKASU, T.: Differentialkugelgeometrie III, The Science Reports of the Tôhoku Imp. Univ. Vol. XXI (1932) p. 600.
- (2) BLASCHKE, W.: Vorlesungen über Differentialgeometrie II (1923), Berlin, S. 156, S. 163, S. 165, S. 162, S. 218.

$$(6) \quad \begin{cases} x_{ik} = A_{ik} \{-D_i \varphi_s - N_i x\} + \frac{1}{2} G_{ik} x, \\ \hat{\varepsilon} = \frac{1}{G^{\frac{1}{2}}} \{-D_i \varphi_s - N_i x\} \times \{-D_i \varphi_s - N_i x\}, \end{cases}$$

weil

$$(7) \quad x = \frac{1}{2} x$$

besteht.

Die Affinentfernung eines Punktes z der Flächenstelle ist mit

$$(8) \quad p = \frac{(z - x, -D_u \varphi_s - N_u x, -D_i \varphi_s - N_i x)}{(LN - M)^{\frac{1}{2}}}$$

gegeben.

Wenn die v -Linien ($u = \text{const.}$) gerade sind, so wird

$$(9) \quad (x_r \times x_u) = \{-D_i \varphi - N_i x\} \times \{A_{ir} x_i + G_{ur} u\} = 0.$$

Aus den Ableitungsgleichungen Weingartens werden

$$(10) \quad \eta_u = -H_{xu} + \frac{A_{ur}}{F^2} x_r, \quad \eta_i = -H_{xi},$$

wo (3) bestehen.

Wenn auf zwei Flächen

$$x \quad \text{und} \quad \bar{x},$$

ihre gemeinsamen Tangenten ein Normalensystem bilden, so folgt

$$\sigma x_{uv} + \sigma x_u + x_v = 0,$$

wo (3), (6) bestehen.⁽¹⁾

Nehmen wir θ anstatt x , so entstehen die folgenden Ableitungsgleichungen.⁽²⁾

- (1) Vergl. NAKAJIMA, S.: Über zwei Flächen, welche eine Beziehung haben Tôhoku Math. Journ. Vol. 30 (1928) p. 142.
 (2) NAKAJIMA, S.: op. cit.

$$\varphi_{hk} = -g_{hk}\varphi + \bar{D}_{hk}\theta + D_{hk}\bar{\theta},$$

$$\theta_h = -D'_h\varphi_s - N_h\theta,$$

$$\bar{\theta}_h = -\bar{D}'_h\varphi_s + N_h\bar{\theta}.$$

Aus⁽¹⁾

$$\theta = \cos\tau \cdot \mathfrak{E} + \sin\tau \cdot \delta + i\eta,$$

$$\theta_s = -\sin\tau \cdot \mathfrak{E} + \cos\tau \cdot \delta,$$

$$\begin{aligned} \theta_t = & -A\cos\tau \mathfrak{A} - B\sin\tau \mathfrak{B} - (S\sin\tau + iT)\mathfrak{E} \\ & + (S\cos\tau - iV)\delta + (T\cos\tau + V\sin\tau)\eta, \end{aligned}$$

kann man

$$\eta, \mathfrak{A}, \mathfrak{B}, \delta, \text{ u. s. w.}$$

rechnen.

Mit

$$\tau = \tan^{-1} \frac{(\partial\theta)}{(\mathfrak{E}\theta)} = \text{const.}$$

oder

$$t = \eta^{-1} \{J\theta\}_t = \text{const.}$$

bezeichnen wir die Kreischaren auf θ .

(3) Über Stützfunktion

Es sei $p(\theta)$ die Stützfunktion einer konvexen Kurve k im R.

Die Kreise $\hat{\xi}(\theta)$ sind die Schmiegekreise der Kurve k .

Für die Kurve ist

$$(1) \quad (\dot{\xi}(\theta) \dot{\xi}(\theta)) = 1.$$

$\hat{\xi}(\theta)$ und $\hat{\xi}(\theta + \pi)$ sind die Gegenpunkte von k .

Gehen die Schmiegekreise $\hat{\xi}(\theta)$ und $\hat{\xi}(\theta + \pi)$ durch den Punkt $\hat{\xi}(\theta + \pi)$ bzw. $\hat{\xi}(\theta)$ hin, so folgt

(1) NAKAJIMA, S.: Kugelgeo. von MÖBIUS, Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ. Vol. II (1929) p. 86.

$$(2) \quad \left\{ \begin{array}{l} (\dot{\xi}(\theta + \pi) \hat{\xi}(\theta)) = 0, \\ (\dot{\xi}(\theta) \hat{\xi}(\theta + \pi)) = 0. \end{array} \right.$$

Aus (2) folgt

$$(3) \quad (\dot{\xi}(\theta + \pi) \hat{\xi}(\theta)) + (\dot{\xi}(\theta) \hat{\xi}(\theta + \pi)) = 0,$$

so folgt

$$(4) \quad (\dot{\xi}(\theta + \pi) \hat{\xi}(\theta)) = \text{const.},$$

d. h. der Winkel zwischen $\dot{\xi}(\theta + \pi)$ und $\hat{\xi}(\theta)$ ist konstant.

Also folgt der

Satz: *Gehen die Schmiegkreise in einer Eilinie immer durch die Gegenpunkte hin, so ist der Winkel zwischen zwei Schmiegkreisen immer konstant.*

Betrachten wir zwei Punkte $\hat{\xi}(\theta)$, $\hat{\xi}(\theta + a)$ anstatt $\hat{\xi}(\theta)$, $\hat{\xi}(\theta + \pi)$, so besteht auch der obige Satz, wo a eine Konstante ist.

Seien η die festen Kreise im R_s , so ergibt sich:

$$(5) \quad (\dot{\xi}(\theta) \cdot \eta) = 0,$$

wo $\dot{\xi}(\theta)$ die Schnittpunkte von η mit k bedeuten.

Aus (5) folgt

$$(\dot{\xi} \eta) = \text{const.},$$

so folgt der

Satz: *Wenn in den Schnittpunkten von K mit den festen Kreisen η die Winkel zwischen den Schmiegkreisen und η immer konstant sind, so sind die Winkel zueinander gleich.*

Wenn ein fester Punkt \mathfrak{A} auf den Schmiegkreisen von K liegt, so folgt

$$(\mathfrak{A} \dot{\xi}) = 0,$$

so folgt

$$(\mathfrak{A} \hat{\xi}) = 0.$$

Wir erhalten also den Satz:

Dasz jeder Schmiegekreis von K durch einen festen Punkt \mathfrak{A} immer hindurchgehe, so musz \mathfrak{A} auf K sein.

Wenn jeder Schmiegekreis $\hat{\xi}$ mit einem festen Kreise η einen festen Winkel enthält, so folgt

$$(\eta \hat{\xi}) = \text{const.},$$

so folgt

$$(\eta \dot{\hat{\xi}}) = 0,$$

also muß der Punkt von k auf η liegen.

Es sei $\hat{\xi}(t)$ die einparametrische Kreisschar im R_2 , $\dot{\hat{\xi}}(t)$ mit $\dot{\hat{\xi}}=0$, die Stellung der zugehörigen Tangentenelemente. Wenn wir die Ableitungen nach dem Parameter t mit den Punkten bezeichnen, so lautet die Bedingung dafür, dass zwei Nachbarkreise $\hat{\xi}$ die Kurve $\dot{\hat{\xi}}$ berühren,

$$(6) \quad \dot{\hat{\xi}} \dot{\hat{\xi}} = 0.$$

Wir können die Streifenbedingung (1) durch

$$(7) \quad \hat{\hat{\xi}}(t) = \hat{\xi}(t) + \lambda(t) \dot{\hat{\xi}}(t)$$

ersetzen, weil die Bedingung (1) auch für $\hat{\hat{\xi}}$ erfüllt ist.

Es ist ja

$$(8) \quad \hat{\hat{\xi}} \dot{\hat{\xi}} = (\dot{\hat{\xi}} + \lambda \ddot{\hat{\xi}} + \lambda \dot{\hat{\xi}}) \dot{\hat{\xi}} = 0$$

vermöge

$$\dot{\hat{\xi}} \dot{\hat{\xi}} = 0.$$

Wir wollen nun dem willkürlichen Faktor $\lambda(t)$ in (7) einen bestimmten Wert erteilen.

Wenn ein Kreis η zu $\hat{\xi}$ senkrecht ist, so folgt

$$(9) \quad \eta \hat{\hat{\xi}} = \hat{\xi} \eta + \lambda \dot{\hat{\xi}} \eta = 0,$$

wo wir λ als die Konstante betrachten.

Daraus folgt

$$(10) \quad \lambda \equiv -\frac{(\dot{\xi} \eta)}{(\dot{\xi} \dot{\eta})}$$

Aus (7) und (10) ergibt sich:

$$(11) \quad \hat{\xi} = \dot{\xi} - (\xi \eta / (\dot{\xi} \dot{\eta})) \dot{\xi}.$$

Der Parameter t soll durch einen neuen Parameter s ersetzt werden, wo s definiert ist durch die Forderung

$$(11) \quad s = \int \pm \sqrt{\dot{\xi}^2} dt + c \\ = \int \pm \sqrt{\dot{\xi}^2 - \frac{(\dot{\xi} \eta)}{(\dot{\xi} \dot{\eta})} \dot{\xi}} dt + c.$$

(4) Geometrischer Ort von einem Punkt, dem Berührungspunkt zweier Kreise

Man betrachte eine ebene Kurve K als einen geometrischen Ort von einem Punkt ξ , dem Berührungspunkt zweier Kreise ξ , η im R_2 , so folgt

$$(1) \quad \xi(t) = \dot{\xi}(t) - (\dot{\xi}(t), \eta(t)) \eta(t),$$

wo

$$(1') \quad (\dot{\xi}(t), \eta(t))^2 = 1,$$

wo t ein Parameter ist.⁽¹⁾

Nach elementarer Differentialgeometrie weisz man, dass die Bogenlänge gegeben ist mit

$$(2) \quad s = \int_a^t \sqrt{(\xi')^2} dt \\ = \int_a^t \sqrt{(\dot{\xi} - \{(\dot{\xi} \eta) + (\dot{\xi} \dot{\eta})\} \eta - (\dot{\xi} \eta) \dot{\eta})^2} d\tau,$$

wo (1') besteht.

(1) THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ., IV, Bd. (1925) S. 122.

Man betrachte

$$s=s(t),$$

so erkennt man, dass man statt des Parameters t längs unserem Kurvenbogen die Bogenlänge s als Parameter einführen kann.

Dann wird

$$(3) \quad \xi(s) = \xi(s) - (\xi(s), \eta(s)) \eta(s), \quad (\xi \eta)^2 = 1.$$

Ist daher $\vartheta(s)$ der Winkel, um den man im positiven Sinn den Einheitsvektor e_1 der x_1 -Achse zu drehen hat, um ihn in den Tangentenvektor $\dot{\xi}(s)$ im Punkte s der Kurve

$$\begin{aligned} \xi &= \xi(s) \\ &= \xi(s) - \eta(s) (\xi \eta), \quad (\xi \eta)^2 = 1 \end{aligned}$$

überzuführen, und bedeutet s die Bogenlänge, so wird man in

$$\kappa = \frac{d\vartheta}{ds}$$

ein Maß für die gesuchte Abweichung vom geradlinigen Verlauf haben.

Wir nennen κ die Krümmung der orientierten Kurve im Punkte s . Die Krümmung des Kreises vom Radius r ist mit

$$\kappa = 1/r$$

gegeben.

Zu $\xi(t)$ oder $\xi(s)$ in (1) oder (2) kann man die Formeln in elementarer Differentialgeometrie anwenden und daher kommt:

$$[1] \text{ Tangentenvektor: } \dot{\xi} = \frac{d\xi}{dt}.$$

$$[2] \text{ Bogenlänge: } s = \int \sqrt{\{\dot{\xi}'(t)\}^2} dt.$$

$$[3] \text{ Einheitsvektor der Tangente: } v_1 = \dot{\xi} = \frac{d\xi}{ds}.$$

$$[4] \text{ Krümmungsradius: } \rho = 1/\kappa, \quad \kappa \neq 0.$$

[5] *Krümmungsmittelpunkt*: $\eta = \xi(s) + \rho v_2(s)$, wo v_2 Einheitsvektor der Normalen ist.

[6] *Evolute*: Geometrischer Ort der Krümmungsmittelpunkte.

Setzen wir

$$\xi(u^1, u^2) = \xi(u^1, u^2) - (\xi(u^1, u^2), \eta(u^1, u^2)) \cdot (\xi \eta)^{\perp} = 1,$$

wo ξ, η zwei Kugeln im R_3 , u^i Parameter sind, so kann man mit $\xi(u^1, u^2)$ eine Fläche bestimmen.

(1) *Erste Fundamentalform*: $\dot{\xi}^2 = \sum g_{ik} \dot{u}^i \dot{u}^k = g_{ik} \dot{u}^i \dot{u}^k$.

(2) *Winkel θ zweier Tangentialvektoren*:

$$\cos \theta = g_{ik} \dot{u}^i \dot{u}^k / \sqrt{g_{ik} \dot{u}^i \dot{u}^k} \sqrt{g_{ik} \dot{u}^i \dot{u}^k},$$

$$\sin \theta = (\dot{u}^1 \dot{u}^2 - \dot{u}^2 \dot{u}^1) / \sqrt{g_{11} g_{22} - g_{12}^2} \sqrt{g_{ik} \dot{u}^i \dot{u}^k} \sqrt{g_{ik} \dot{u}^i \dot{u}^k}.$$

(3) *Oberfläche*:

$$\iint \sqrt{g_{11} g_{22} - g_{12}^2} du^1 du^2.$$

(4) *Zweite Fundamentalform*:

$$\xi \xi_{,3} = L_{ik} \dot{u}^i \dot{u}^k,$$

$$\xi_{,ik} = \frac{\partial^2 \xi}{\partial u^i \partial u^k},$$

$$L_{ik} = \xi_{,ik} \xi_3 = -\xi_i \xi_{,k3}.$$

(5) *Krümmung des Normalschnittes*:

$$\bar{k} = L_{ik} \dot{u}^i \dot{u}^k / g_{ik} \dot{u}^i \dot{u}^k.$$

(6) *Formel von Meusnier*:

$$k \cos \vartheta = \bar{k}.$$

(7) *Hauptkrümmungen*: \bar{k}_1, \bar{k}_2 definiert als Extremwert von \bar{k} .

Sie genügen der quadratischen Gleichung $\bar{k}^2 - 2H\bar{k} + k = 0$.

(8) *Mittlere Krümmung*:

$$H = \frac{1}{2} (\bar{k}_1 + \bar{k}_2) = \frac{1}{2} (g_{11}L_{22} - g_{12}L_{12} + g_{22}L_{11}) / (g_{11}g_{22} - g_{12}^2).$$

{9} GAUSZsche Krümmung:

$$K = \frac{L_{11}L_{22} - L_{12}^2}{g_{11}g_{22} - g_{12}^2} = \bar{k}_1\bar{k}_2 = L_1^1 L_2^2 - L_1^2 L_2^1.$$

{10} Differentialgleichung der Krümmungslinien:

$$\begin{vmatrix} L_{1k}\dot{u}^k & g_{1k}\dot{u}^k \\ L_{2k}\dot{u}^k & g_{2k}\dot{u}^k \end{vmatrix} = 0$$

oder

$$\begin{vmatrix} \dot{u}^2\dot{u}^2 & -\dot{u}^1\dot{u}^2 & \dot{u}^1\dot{u}^1 \\ g_{11} & g_{12} & g_{22} \\ L_{11} & L_{12} & L_{22} \end{vmatrix} = 0.$$

{11} Die Krümmungslinien sind dann und nur dann Parameterlinien, wenn $g_{12} = L_{12} = 0$ ist.

{12} CHRISTOFFELSche Symbole:

Die erster Art: $[\begin{smallmatrix} ik \\ i \end{smallmatrix}] = \frac{1}{2} \left(\frac{\partial g_{ii}}{\partial u^k} + \frac{\partial g_{ki}}{\partial u^i} - \frac{\partial g_{ik}}{\partial u^i} \right).$

Die zweiter Art: $\{\begin{smallmatrix} ik \\ i \end{smallmatrix}\} = g'^m [\begin{smallmatrix} ik \\ m \end{smallmatrix}]$.

{13} Ableitungsformeln:

Die von WEINGARTEN: $\xi_{ik} = \{\begin{smallmatrix} ik \\ i \end{smallmatrix}\} \xi_i + L_{ik} \xi_3, \quad i, k = 1, 2$

Die von GAUSZ: $\xi_{3i} = -L_i^k \xi_k, \quad i, k = 1, 2$

$$L_i^k = g^{kj} L_{ij}$$

{14} Fundamentalgleichungen:

Die von Codazzi-Mainardi:

$$\begin{aligned} \frac{\partial L_{12}}{\partial u^1} - \frac{\partial L_{11}}{\partial u^2} + \left\{ \begin{smallmatrix} 12 \\ l \end{smallmatrix} \right\} L_{11} - \left\{ \begin{smallmatrix} 11 \\ l \end{smallmatrix} \right\} L_{12} &= 0 \\ \frac{\partial L_{22}}{\partial u^1} - \frac{\partial L_{21}}{\partial u^2} + \left\{ \begin{smallmatrix} 22 \\ l \end{smallmatrix} \right\} L_{11} - \left\{ \begin{smallmatrix} 21 \\ l \end{smallmatrix} \right\} L_{12} &= 0 \end{aligned}$$

Die von Gauss: Darstellung des Krümmungsmasses K durch die g_{ik} :

$$\begin{aligned} \sqrt{g_{11}g_{22}-g_{12}^2} K = & \frac{\partial}{\partial u^2} \left(\frac{\sqrt{g_{11}g_{22}-g_{12}^2}}{g_{11}} \left\{ \begin{matrix} 11 \\ 2 \end{matrix} \right\} \right) \\ & - \frac{\partial}{\partial u^1} \left(\frac{\sqrt{g_{11}g_{22}-g_{12}^2}}{g_{11}} \left\{ \begin{matrix} 12 \\ 2 \end{matrix} \right\} \right) \end{aligned}$$

und

$$\begin{aligned} \sqrt{g_{11}g_{22}-g_{12}^2} K = & - \frac{\partial}{\partial u^2} \left(\frac{\sqrt{g_{11}g_{22}-g_{12}^2}}{g_{22}} \left\{ \begin{matrix} 21 \\ 1 \end{matrix} \right\} \right) \\ & + \frac{\partial}{\partial u^1} \left(\frac{\sqrt{g_{11}g_{22}-g_{12}^2}}{g_{22}} \left\{ \begin{matrix} 22 \\ 1 \end{matrix} \right\} \right) \end{aligned}$$

Definition: Kurven stationärer Länge. Extremalen des Variationsproblems: $\int \sqrt{\xi^2} dt = \text{stationär}$, wobei $u^i f_i(t)$,

$$\xi(t) = \xi(t) - \eta(t) (\xi \eta), \quad (\xi \eta)^2 = 1.$$

(5) Zweiparametrische Kugel- und Kreisscharen

(1)

Hier stelle ich eine Theorie der zweiparametrischen Kreisscharen im konformen Raum auf.

Ist eine Kreisschar im konformen Raume vorgelegt, dann gibt es ein Paar Örter des Brennpunktes der Kreise.

Diese Örter sind ja eine Fläche der zweiparametrischen Kreisscharen. Sind die Gleichungen eines Paares Örter:

$$(1) \quad (\xi \xi) \equiv x_0^2(u, v) + x_1^2(u, v) + x_2^2(u, v) + x_3^2(u, v) = 0,$$

$$(2) \quad (\eta \eta) \equiv y_0^2(u, v) + y_1^2(u, v) + y_2^2(u, v) + y_3^2(u, v) = 0,$$

dann kann man die Gleichung der Kreisscharen als

$$(3) \quad (\xi \eta) \equiv x_0 y_0 + x_1 y_1 + x_2 y_2 + x_3 y_3 = 1$$

setzen, wobei u, v die Parameter bedeuten.

Jetzt kann man drei quadratische Differentialformen

$$(4) \quad \begin{cases} \sum dx_i dy_i = G_{ij} du^i du^j, \\ \sum (dx^i)^2 = g_{ij} du^i du^j, \\ \sum (dy_i)^2 = \bar{g}_{ij} du^i du^j \end{cases}$$

betrachten, wo dx_i , dy_i zwei gegebene Fortschreitungsrichtungen bedeuten.⁽¹⁾

Drei quadratische Formen G_{ij} , g_{ij} , \bar{g}_{ij} haben fünf simultane Invarianten, deren Repräsentanten wir unter Zugrundelegung von G_{ij} als Fundamentalformen folgendermassen schreiben können:

$$(5) \quad \begin{cases} \frac{1}{2} G_{ik} g_{ik} = h, & \frac{g}{G} = k, e^{11} = 0, e^{12} = \frac{1}{\sqrt{G}}, e^{21} = -\frac{1}{\sqrt{G}}, e^{22} = 0, \\ \frac{1}{2} G_{ik} \bar{g}_{ik} = \bar{h}, & g = |g_{ij}|, G = |G_{ij}|, \bar{g} = |\bar{g}_{ij}|, \\ \frac{i}{2} e^{ik} G^{pq} g_{ip} \bar{g}_{kq} = d, & \frac{\bar{g}}{G} = \bar{k}, \quad (i = \sqrt{-1}) \end{cases}$$

Man kann die Formel leicht nachweisen:

$$(6) \quad \frac{g}{G} = -\frac{1}{2} e^{ik} e^{pq} g_{ip} g_{kq},$$

$$(7) \quad G^{rs} g_{ij} g_{ks} = 2h g_{ik} - k g_{ik}.$$

Analoge Gleichungen erhält man durch Vertauschung von g_{ik} mit \bar{g}_{ik} . Neben zwei der Formen gibt es noch eine sogenannte JACOBISCHE Form.

Die drei Jacobischen Formen sind folgendes:

$$p_{ik} = \frac{1}{2} (e_{ir} g_{ik}^r + e_{kr} g_{ir}^r),$$

$$\bar{p}_{ik} = \frac{1}{2} (e_{ir} \bar{g}_{ik}^r + e_{kr} \bar{g}_{ir}^r),$$

(1) NAKAJIMA, S.: Differentialgeometrie der Kreisscharen, X, XI, XII, Tôhoku Math. Journ. Vol. 34 (1931) p. 191.

$$f_{ik} = -\frac{i}{2} e_{rs} (g^s \bar{g}^k - g^k \bar{g}^s)$$

Es gelten folgende Gleichungen :

$$G^{ik} p_{ik} = 0, \quad G^{ik} \bar{p}_{ik} = 0,$$

$$g^{ik} p_{ik} = 0, \quad \bar{g}^{ik} \bar{p}_{ik} = 0,$$

$$-\frac{i}{2} e^{ik} G^{pq} p_{ip} \bar{p}_{kq} = d,$$

$$G^{ik} f_{ik} = d.$$

Wir denken uns die Kugeln des euklidischen R_3 in bekannter Weise auf die Punkte des euklidischen R_4 abgebildet, indem wir der Kugel mit der Gleichung

$$(x-a)^2 + (y-b)^2 + (z-c)^2 = r^2$$

den Punkt mit den Koordinaten $a, b, c \pm ir$ zuordnen.

Den beiden Werten von ir tragen wir dadurch Rechnung, dass wir die Kugel durch die Angabe eines Drehungssinnes auf ihrer Oberfläche orientieren.

Einer Kugel mit positivem Drehungssinn soll das positive, einer Kugel mit negativem Drehungssinne das negative Zeichen zugeordnet werden.

Wir sagen kurz, die Punkte des R_4 sind isotrop auf die orientierten Kugeln des R_3 projiziert.

(2)

Sollen sich zwei Kugeln mit den Koordinaten (a_1, b_1, c_1, r_1) , (a_2, b_2, c_2, r_2) gleichsinnig berühren, so muss

$$(1) \quad (a_1 - a_2)^2 + (b_1 - b_2)^2 + (c_1 - c_2)^2 = (r_1 - r_2)^2$$

sein, d. h. die beiden Bildpunkte im R_4 müssen die Entfernung Null haben.

Nun setzen wir

$$a \equiv x_1, \quad b \equiv x_2, \quad c \equiv x_3, \quad ir_3 \equiv x_4,$$

dann kann man mit $x(u^1, u^2)$ zweidimensionale Mannigfaltigkeit im Raume von vier Dimensionen bezeichnen, wo u^i zwei Parameter sind.

Setzen wir

$$\frac{\partial x}{\partial u^i} = x_i,$$

so bilden zwei entsprechende Koordinaten von x_1 und x_2 einen kovarianten Vektor.

Es ist nun

$$(2) \quad (dx \, dx) = (x_i \, x_k) du^i du^k = g_{ik} du^i du^k$$

eine parameterinvariante Form.

Die Nulllinien unserer Form sind die Minimallinien im R_4 .

Die Minimallinien im R_4 entsprechen den Kugelscharen im R_3 , so folgt der

Satz: Betrachten wir ∞^2 Kugeln $\eta(u^1, u^2)$ im R_3 , so berühren sie sich gleichsinnig durch eine feste Kugel $\eta(u_0^1, u_0^2)$ in zwei Richtungen der Kugeln.

(3)

Es seien eine Kugelkongruenz

$$\varphi = \varphi(u^1, u^2),$$

$$(\varphi\varphi) = 1$$

und deren beide Enveloppenmäntel

$$\bar{x} = \bar{x}(u^1, u^2), \quad ((\bar{x} \, \bar{x}) = 0)$$

$$\bar{\bar{x}} = \bar{\bar{x}}(u^1, u^2), \quad ((\bar{\bar{x}} \, \bar{\bar{x}}) = 0)$$

gegeben. Man führe die Bezeichnung

$$\frac{\partial \varphi}{\partial u^i} = \varphi_i$$

ein. Die betreffenden Koordinaten seien wie folgt normiert:

$$(\xi \bar{\xi}) = 1.$$

Die quadratische Form

$$\partial \xi = (\xi_i \partial u^i) (\xi_k \partial u^k) = g_{ik} \partial u^i \partial u^k,$$

$$g_{ik} = \xi_i \bar{\xi}_k$$

ist positiv definit und dem gewöhnlichen Bogenelement der Fläche (ξ) proportional.

Wegen der linearen Unabhängigkeit von $\xi, \eta, \xi, \varphi, \bar{\xi}$ nach BLASCHKE⁽¹⁾ können wir die zweiten kovarianten Ableitungen von ξ bezüglich $\partial \xi^k$ linear aus ihnen zusammensetzen und finden

$$\xi_{ik} = r_{ik} \xi + s_{ik} \varphi - g_{ik} \bar{\xi}.$$

Darin ist $\xi_{ik} \bar{\xi}_i = 0$ und

$$g_{ik} = \xi_i \bar{\xi}_k = -\bar{\xi} \xi_{ik},$$

$$r_{ik} = \xi_{ik} \bar{\xi} = -\bar{\xi}_i \bar{\xi}_k = -\bar{\xi}_k \bar{\xi}_i,$$

$$s_{ik} = \xi_{ik} \varphi = -\bar{\xi}_i \varphi_k = -\bar{\xi}_k \varphi_i.$$

(4)

Hier wollen wir die Geometrie im elliptischen Raum studieren. Es seien x_0, x_1, x_2, x_3 die homogenen Koordinaten eines Punktes ξ im projektiven Raume.

Wir können sie für reelle Punkte immer und zwar auf zwei Arten so nomieren, dass

$$(1) \quad (\xi \xi) \equiv x_0^2 + x_1^2 + x_2^2 + x_3^2 = 1$$

wird. Dann sind die Punkte x_0, x_1, x_2, x_3 und $-x_0, -x_1, -x_2, -x_3$ miteinander identisch.

Die Entfernung φ zweier Punkte ξ, η können wir in den normierten Koordinaten durch die Formel erklären :

(1) BLASCHKE, W.: Über konforme Geometrie IV, Abh. aus dem Math. Seminar der Hamb. Univ. Bd. IV (1926), S. 226.

$$(2) \quad \cos \varphi = x_0 y_0 + x_1 y_1 + x_2 y_2 + x_3 y_3 \equiv (x y)$$

Dann lassen die eigentlich orthogonalen Substitutionen der x_i, y_i die Formen $(x x)$, $(y y)$ und die Polarenbildung $(x y)$ invariant; wir können sie also als Bewegungen des elliptischen Raumes mit Maszbestimmung (2) bezeichnen.

Wir betrachten ein ein-parametrisches System

$$(3) \quad x = x(s),$$

zwei benachbarte Kurvensysteme (3) haben den Abstand ds .

$$(4) \quad \cos(ds) = (dx dx);$$

demnach wird

$$(5) \quad (dx dx) = 1.$$

Aus (5) ergibt sich

$$(6) \quad (d^2 x dx) = 0, (d^3 x dx) = (d^2 x d^2 x), \text{ u. s. w.}$$

Drei konsekutive Punkte bestimmen das Feld

$$(7) \quad x = \rho || \ddot{x} \ddot{x} ||^{\frac{1}{2}},$$

wo ρ einen Proportionalitätsfaktor zeigt.

$$(8) \quad (x y) = 0$$

bedeutet, der Abstand sei $\pi/2$ gleich.

$$(9) \quad (x x)(y y) - (x y)^2 = 0$$

bedeutet, der Abstand sei Null gleich.

Wenn $\varphi = \xi + r y$ ist, so liegt der Punkt φ auf der Verbindungsgerade von zwei Punkten ξ und y .

Wenn ϕ der Abstand zwischen φ und x ist, so ergibt sich

$$(10) \quad \cos \phi = (x, \xi + r y),$$

wo r eine Konstante ist.

Man berechnet nach (2) für den unendlich kleinen Abstand $d\psi$ zwischen einem Punkt ξ und dem Nachbarpunkt $\xi + \dot{\xi} dt$

$$(11) \quad \tan^2 d\psi = d\psi^2 = d\sigma^2.$$

Wenn wir einen Punkt ξ als die Funktion eines Parameters t ansehen, so ist eine Kurve dadurch in der Ebene bestimmt.

Es gelten dann nach (1)

$$(12) \quad (\xi \xi) = 1 \quad \text{und} \quad (\xi \xi')$$

identisch in t , wenn wir die Ableitungen nach t durch den Strich bezeichnen.

Zu den Kurven im elliptischen Raume wendet man STRANSKY'S⁽¹⁾ und KUBOTA'S⁽²⁾ Arbeit an.

Ist ds das durch das Feld \mathfrak{X} geschriebene Bogenelement, so wird

$$(13) \quad \cos(ds) = (d\mathfrak{X} d\mathfrak{X}).$$

Ein Punkt \mathfrak{z} , als die Funktion $\mathfrak{z}(u^1, u^2)$ zweier Parameter u^1 und u^2 betrachtet, beschreibt eine Fläche.

Es gilt $(\mathfrak{z} \mathfrak{z}) = 1$ identisch in u^i .

Es ist nun

$$1 = (d\xi d\mathfrak{z}) = \xi_i \mathfrak{z}_k du^i du^k = g_{ik} du^i du^k.$$

Wir definieren den Abstand θ zwischen zwei Punkten von v^i, w^i mit

$$\cos \theta = \frac{g_{\lambda\mu} v^\lambda w^\mu}{\sqrt{g_{\lambda\mu} v^\lambda v^\mu} \sqrt{g_{\lambda\mu} w^\lambda w^\mu}}.$$

Setzen wir

$$\begin{cases} \delta g^{\lambda\nu} = Q^{\cdot\lambda\nu} dx^\mu, \\ V_\mu g^{\lambda\nu} = Q^{\cdot\lambda\nu}_\mu, \end{cases}$$

so folgt

$$\delta g_{\lambda\nu} = -g_{\lambda\alpha} g_{\nu\beta} Q^{\alpha\beta}_\mu dx^\mu.$$

$$V_\mu g^{\lambda\nu} = -g_{\lambda\alpha} g_{\nu\beta} Q^{\alpha\beta}_\mu,$$

(1) STRANSKY, E.: Zur Infinitesimalgeo. der Kurven im elliptischen Raume, Sitzungsberichten der kaiserl. Akademie der Wiss. in Wien Bd. CXXI (1912) S. 1.

(2) KUBOTA, T.: Differentialgeo. in noneuklidischen Raum, Tōkyō Buzuri-gakko Zassi Vol. 31, p. 128.

$$\begin{aligned}Q_{\mu}^{\lambda\nu} &= Q_{\mu} g^{\lambda\nu}, \\ \delta g^{\lambda\nu} &= Q_{\mu} g^{\lambda\nu} dx^{\mu}, \\ V_{\mu} g^{\lambda\nu} &= Q_{\mu} g^{\lambda\nu},\end{aligned}$$

so folgt

$$\begin{aligned}\delta g_{\lambda\nu} &= -Q_{\mu} g_{\lambda\nu} dx^{\mu}, \\ Q^{\cdot}{}_{\lambda\mu\nu} &\equiv V_{\mu} g_{\lambda\nu} = -g_{\lambda\sigma} g_{\nu\rho} Q_{\mu}^{\sigma\rho}.\end{aligned}$$

In unserem Falle ist

$$g_{\lambda\mu} du^{\lambda} du^{\mu} = 1 = \bar{g}_{\lambda\mu} d\bar{x}^{\lambda} d\bar{x}^{\mu},$$

also folgt

$$g_{\lambda\mu} = g_{\omega\nu} \frac{\partial u^{\omega}}{\partial \bar{u}^{\lambda}} \frac{\partial u^{\nu}}{\partial \bar{u}^{\mu}}.$$

(5)

Ein Punkt ξ , als die Funktion $\xi(u, v)$ zweier Parameter u, v betrachtet, beschreibt eine Fläche, so folgt

$$(1) \quad (\dot{\xi} \dot{\xi}) = 0, \quad (\hat{\xi} \hat{\xi}) = 1,$$

wo ξ eine Kugel bedeutet.⁽¹⁾

Hier betrachten wir die Kugelflächen und nehmen etwa die Kugel⁽¹⁾

$$(2) \quad \dot{\xi} + \hat{\xi},$$

so ergibt sich⁽²⁾

$$(3) \quad \ddot{\xi} + \dot{\xi} = 0,$$

wo

- (1) THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 126.
- (2) THOMSEN, G.: op. cit.

$$\dot{\xi} = -\frac{\partial \xi}{\partial u}$$

ist.

Aus (1) folgt

$$(4) \quad \xi(u, v) = -f(v)e^{-u} + \varphi(v),$$

wo

$$(\xi \xi) = 1$$

besteht.

Aus (4) ergibt sich:

$$\xi = Ve^{-u} + \phi(V)$$

wo u, V zwei Parameter sind.

(6)

Sind drei verschiedene Kugeln ξ, η, φ im R_3 gegeben, so darf man sagen: Beliebige Kugeln ϑ, σ im R_3 sollen die Gleichung

$$B_{\mu\nu} \vartheta^\mu \sigma^\nu =, \xi, \eta, \varphi, \vartheta, \sigma |$$

identisch erfüllen, was ein eindeutig bestimmter Satz von Grössen $B_{\mu\nu}$ ist, den wir als Punktepaar im R_3 bezeichnen.

Die Grössen $B_{\mu\nu}$ nennen wir seine Koordinaten.

Insbesondere nennen wir das Punktepaar in bezug auf die Kugeln ξ, η, φ ihre Schnittpunkte, wo

$$|| \xi, \eta, \zeta || \neq 0$$

besteht.

(7)

$\xi(u, v)$ sei die Kugel im R_3 der zu verbiegenden Kugel

$$(1) \quad \xi_i = \xi + 2t\delta + 2t^2\delta^{(2)} + \dots,$$

wo die δ auch von u, v abhängig sind, so stellt $\xi(u, v)$ eine Verbiegung bestimmter Stufe dar, wenn die Entwicklung nur bis zu der

entsprechenden Ordnung berücksichtigt wird und die Gleichung

$$d\xi_1^2 = d\xi^2$$

bis zu den Gliedern dieser Ordnung erfüllt ist.

Wenn wir statt dessen schreiben :

$$(2) \quad d(\xi_1 - \xi) \cdot d(\xi_1 + \xi) = 0,$$

so folgt

$$(3) \quad d(t_\beta + t_\beta^{(2)} + \dots) \cdot d(\xi + t_\beta + t_\beta^{(2)} + \dots) = 0.$$

Darin sind folgende Gleichungen

$$(4) \quad \begin{cases} d\xi d\beta = 0, \\ d\xi d\beta^{(2)} + d\beta^{(2)} = 0, \\ d\xi d\beta^{(3)} + d\beta d\beta^{(2)} + d\beta^{(2)} d\beta = 0, \quad \text{u. s. w.,} \end{cases}$$

Nach (4₁) kann man sagen, dass $d\beta$, $d\xi$ zueinander senkrecht sind.

(6) Koordinaten von Gauss

Führen wir in unsere Möbius-Ebene nach Gauss statt der kartesischen Koordinaten ξ , η die komplexe Veränderliche

$$(1) \quad z = \xi + i\eta \quad i = \sqrt{-1}$$

ein, so ergibt sich :

$$(2) \quad \begin{cases} x_0 = \sigma(1 + z\bar{z}), \\ x_1 = \sigma(1 - z\bar{z}), \\ x_2 = \sigma(z + \bar{z}), \\ x_3 = -i\sigma(z - \bar{z}), \end{cases}$$

wo

$$(3) \quad \bar{z} = \xi - i\eta, \quad z = \frac{x_2 + ix_3}{x_0 + x_1}$$

ist.

Die Gleichung des Kreises ist

$$(4) \quad z\bar{z} = Azz + Bz + \bar{B}\bar{z} + D = 0.$$

Aus der Bedingung

$$(5) \quad z\bar{z} = \frac{4}{r^2} (B\bar{B} - AD)$$

ergibt sich, dass z in (5) einen Punkt bezeichnet, wenn

$$(6) \quad B\bar{B} = AD$$

ist.

Aus (5), (6) folgt

$$(7) \quad ABz\bar{z} + B^2z + A\bar{D}\bar{z} + BD = 0.$$

z in (5) bezeichnet einen Kreis, wenn

$$(8) \quad 4(B\bar{B} - AD) = r^2$$

ist.⁽¹⁾

Aus (3) ergibt sich:

$$(9) \quad \tan \theta = \frac{x_3}{x_2}, \quad r = \sqrt{x_2^2 + x_3^2} / (x_0 + x_1),$$

wo r der absolute Betrag von z und θ die Amplitude von z ist.

Setzen wir

$$(10) \quad \begin{cases} x'_0 = \sigma'(1 + z'\bar{z}'), \\ x'_1 = \sigma'(1 - z'\bar{z}'), \\ x'_2 = \sigma'(z' + \bar{z}'), \\ x'_3 = -i\sigma'(z' - \bar{z}'), \end{cases}$$

so folgt

$$|z + z'| \leq \frac{\sqrt{x_0^2 + x_3^2}}{x_0 + x_1} + \frac{\sqrt{x_2^2 + x_3^2}}{x_0 + x_1}.$$

(1) BLASCHKE, W.: Vorlesungen über Differentialgeometrie III, Berlin (1929) S. 87.

(7) Geometrie von LIE

Hier werden wir die Geometrie von LIE in der Ebene behandeln. Wir wissen, dass wir den gerichteten Kreisen der Ebene eineindeutig die Systeme von vier durch die Bedingung

$$(1) \quad (\xi \eta) = -x_0^2 + x_1^2 + x_2^2 + x_3^2 = 1$$

normierten tetrazyklischen Koordinaten x_i zuordnen können.

Wir setzen

$$(2) \quad x_i = \frac{y_i}{y_4} \quad (i=0, 1, 2, 3)$$

und nennen $y_i (i=0, 1, 2, 3, 4)$ die fünf homogenen pentazyklischen Kreiskoordinaten.

Aus (1), (2) folgt

$$(3) \quad \langle \eta \eta \rangle = -y_0^2 + y_1^2 + y_2^2 + y_3^2 - y_4^2 = 0.$$

Jedem gerichteten Kreis genügt (4).

Wenn $y_4=0$, so bezeichnet (4) einen Punkt.

Die Punkte werden dabei ganz dementsprechend als Kreise mit dem Radius 0 betrachtet, also bezeichnet (3) einen Kreis im R_4 . Wir bezeichnen die zu (3) gehörige aus den pentazyklischen Koordinaten zweier Kreise ξ und η gebildete Bilinearform mit

$$(4) \quad \langle \xi \eta \rangle = -x_0 y_0 + x_1 y_1 + x_2 y_2 + x_3 y_3 - x_4 y_4,$$

oder

$$\xi \eta = -x_0 y_0 + x_1 y_1 + x_2 y_2 + x_3 y_3 - x_4 y_4.$$

Ebenso schreiben wir wieder vektorielle Gleichungen, z. B.:

$$\xi = \alpha \xi + \beta \eta$$

Es gelten für die Produkte mit den eckigen Klammern ganz dieselben Rechen-gesetze wie für die Produkte mit den runden Klammern.

$$\langle \xi \eta \rangle = 0$$

ist die Bedingung für die Berührung zweier Kreise und unsere Lie-Transformationen erhalten die Bedingung der Berührung von K.-Kreisen.

(1)

Ein Satz von drei Kreisen \mathfrak{x}^a sind voneinander linear abhängig. Damit gilt etwa :

$$\mathfrak{x}^{\text{III}} = \alpha \mathfrak{x}^{\text{I}} + \beta \mathfrak{x}^{\text{II}},$$

$$\mathfrak{y}^{\text{III}} = \gamma \mathfrak{y}^{\text{I}} + \delta \mathfrak{y}^{\text{II}},$$

wo $\alpha, \beta, \gamma, \delta$ die skalaren Grössen sind.

Wenn sich $\mathfrak{x}^{\text{III}}, \mathfrak{y}^{\text{III}}$ berühren, so folgt

$$(\mathfrak{x}^{\text{III}} \mathfrak{y}^{\text{III}}) = 0,$$

d. h. $(\alpha \mathfrak{x}^{\text{I}} + \beta \mathfrak{x}^{\text{II}}, \gamma \mathfrak{y}^{\text{I}} + \delta \mathfrak{y}^{\text{II}}) = 0,$

$$\begin{aligned} \alpha \gamma \langle \mathfrak{x}^{\text{I}} \mathfrak{y}^{\text{I}} \rangle + \alpha \delta \langle \mathfrak{x}^{\text{I}} \mathfrak{y}^{\text{II}} \rangle + \\ + \beta \gamma \langle \mathfrak{x}^{\text{II}} \mathfrak{y}^{\text{I}} \rangle + \beta \delta \langle \mathfrak{x}^{\text{II}} \mathfrak{y}^{\text{II}} \rangle = 0, \end{aligned}$$

oder

$$\{\alpha \delta + \beta \gamma\} \langle \mathfrak{x}^{\text{I}} \mathfrak{x}^{\text{II}} \rangle = 0,$$

weil

$$\langle \mathfrak{x}^{\text{I}} \mathfrak{x}^{\text{I}} \rangle = 0, \quad \langle \mathfrak{x}^{\text{II}} \mathfrak{x}^{\text{II}} \rangle = 0$$

bestehen.

Wenn

$$\alpha \delta + \beta \gamma \neq 0,$$

so folgt

$$\langle \mathfrak{x}^{\text{I}} \mathfrak{x}^{\text{II}} \rangle = 0,$$

d. h. zwei K.-Kreise $\mathfrak{x}^{\text{I}}, \mathfrak{x}^{\text{II}}$ müssen sich berühren.

(2)

Wenn b, \bar{b} zwei Kreise sind, so folgt

$$bb=0,$$

$$\bar{b}\bar{b}=0.$$

Wenn b, \bar{b} zwei andere Kreise ξ berühren, so folgt

$$b\xi=0,$$

$$\bar{b}\xi=0.$$

Wenn b, \bar{b} die benachbarten Kreise von ξ berühren, so ergibt sich

$$b\xi'=0,$$

$$\bar{b}\xi'=0.$$

Wir haben jetzt die folgende Tabelle skalarer Produkte zwischen ξ, ξ', b und \bar{b} .

—	ξ	ξ'	b	\bar{b}
ξ	0	0	0	0
ξ'	0	1	0	0
b	0	0	0	1
\bar{b}	0	0	1	0

Aus dem Multiplikationssatz der Determinanten folgt nun

$$|\xi, \xi', b, \bar{b}|^2=0.$$

Also sind die vier in der Determinante stehenden Vektoren voneinander linear abhängig.

(3)

ξ^I, ξ^{II} seien zwei Kugeln, so folgt

$$(\xi^I \xi^I) = 0,$$

$$(\xi^{II} \xi^{II}) = 0.$$

Wenn η, ξ^I, ξ^{II} drei Kugeln, und zwar die K.-Kugeln sind, die sich zusammengehörig berühren, so folgt

$$(1) \quad \eta = \rho_a \xi^a,$$

Wenn sich $\xi^a [a=I, II]$ festhalten, so erhalten wir in (1) ∞' Kugeln von η .

Ist

$$\eta = \rho_a \xi^a$$

eine normierte K.-Kugel mit

$$\eta \eta = \rho_a \rho_a A^{aa} = 0,$$

so muss

$$(2) \quad \cos^2 \varphi = \rho_a \rho_a T^{aa}$$

sein, wo φ den Winkel zwischen η und einem festen K.-Kreis bedeutet.

(4)

Wir können zwei neue Kugeln

$$(1) \quad \xi^a = \sum_{\beta=1}^{II} c_{\beta}^a \xi^{\beta} \quad [a=I, II]$$

als Linearkombinationen der ξ^a einführen mit Koeffizienten c_{β}^a , deren Determinante

$$|c_{\beta}^a| \neq 0$$

sein muss, wenn ξ^{*I} und ξ^{*II} zueinander nicht proportional werden

sollen, und können dann ebensogut durch die ξ^* unsern Kreis darstellen.

ξ^I, ξ^I, ξ^{II} und $\xi^{II}, \xi^I, \xi^{II}$ sollen sich zu dreien zusammengehörig berührende K.-Kugeln sein.

Wir wollen (1) auch Büscheltransformationen der Kugeln nennen.

Für die Behandlung der Geometrie der Kugeln im R_3 erweist es sich als zweckmässig, diese in der angegebenen Weise zunächst durch ganz beliebige Paare von Kugeln darzustellen.

Bilden wir die skalaren Produkte aller dieser Kugeln, so können wir aus ihnen abgesehen von den bekannten Ausnahmen das vollständige Invariantensystem der Figur der gegebenen Kugeln gewinnen;

Bilden wir das System der Skalarprodukte

$$(2) \quad (\xi^a \xi^b) = A^{ab},$$

so haben wir in A^{ab} ein Grössensystem, das sich nach (1) in folgender Weise substituiert:

$$(3) \quad \bar{A}^{ab} = c_a^a c_b^b A^{ab} \quad [\bar{A}^{ab} = (\bar{\xi}^a \bar{\xi}^b)].$$

Hier laufen alle Indizes von I bis II, und doppelt vorkommende Indizes sind auf der rechten Seite zu summieren.

Es wird der Determinante $A = |A^{ab}|$

$$(4) \quad A = |c_a^a|^2 A$$

substituiert.

Zwei orientierte lineare Kongruenzen

$$(5) \quad \begin{cases} (\xi^I \xi) = 0, & ((\xi^I \xi^I) = 1), \\ (\xi^{II} \xi) = 0, & ((\xi^{II} \xi^{II}) = 1) \end{cases}$$

haben die zur pentazyklischen Gruppe gehörige absolute Invariante

$$(6) \quad I_{I,II} = \frac{(\xi^I \xi^{II})}{\sqrt{(\xi^I \xi^I)} \sqrt{(\xi^{II} \xi^{II})}} = \cos W(\xi^I, \xi^{II}),$$

die wir den Kosinus des H.-Winkels $W(a, \beta)$ nennen.

Wollen wir nun eigentliche reelle Kugeln haben, so müssen wir die Determinante

$$A > 0$$

voraussetzen.

Denn diese Gleichung besagt

$$(7) \quad (\xi^I \xi^{II}) (\xi^{II} \xi^{II}) - (\xi^I \xi^{II})^2 > 0,$$

was nach (6) bedeutet, dass die Kugeln ξ^a sich unter dem reellen Winkel schneiden.

Für $A=0$ schrumpft die lineare Kongruenz auf einen Kreis zusammen, für $A<0$ ist er imaginär.

Nun betrachten wir

$$(8) \quad A^{ab} = 0.$$

Dehnen wir unsere LIES Geometrie auf

$$(9) \quad (\xi^I \xi^{II}) = 0$$

aus, so folgt

$$(10) \quad (\xi^I - \xi^{II})^2 + (\eta^I - \eta^{II})^2 + (\zeta^I - \zeta^{II})^2 = -4,$$

d. h. Kugeln im R_3 bezeichnet, weil wir durch Rückgang auf kartesische Koordinaten $\xi^I, \eta^I, \eta^I; \xi^{II}, \eta^{II}, \zeta^{II}$ (10) erhalten aus

$$x_0 = \rho \frac{1 + \xi^2 + \eta^2 + \zeta^2}{2}, \quad x_1 = \rho \frac{1 - (\xi^2 + \eta^2 + \zeta^2)}{2},$$

$$x^2 = \rho \xi, \quad x_3 = \rho \eta, \quad x_4 = \rho \zeta, \quad x_5 = \rho,$$

(ρ Prop. Fakt.).

(5).

Zwei orientierte lineare Kongruenzen

$$(1) \quad \begin{cases} (a \xi)_s = 0, ((a\alpha)_s = 1), \\ (\beta \xi)_s = 0, ((\beta\beta)_s = 1) \end{cases}$$

haben die zur pentazyklischen Gruppe gehörige absolute Invariante.

$$(2) \quad \cos W(\alpha, \beta) = \frac{(\alpha\beta)_s}{\sqrt{(\alpha\alpha)_s(\beta\beta)_s}},$$

oder

$$\cos W(\alpha, \beta) = (\alpha\beta),$$

die wir den Kosinus des H.-Winkels $W(\alpha, \beta)$ nennen.

Man berechnet nach (2) für den unendlich kleinen Winkel zwischen d^h und der Nachbarkongruenz

$$\tan^2 d^h = (\dot{u}\dot{a})dt^2,$$

wo t Parameter und

$$(3) \quad \frac{d\alpha}{dt} = \dot{a}$$

ist.

Da bis auf Glieder von höherer als erster Ordnung

$$\tan(d^\psi) = d^\psi$$

ist, gilt für

$$(4) \quad \begin{aligned} d\sigma &= \sqrt{\dot{a}\dot{a}} dt \\ d\sigma^2 &= d\psi^2. \end{aligned}$$

Wenn \mathfrak{b} , $\bar{\mathfrak{b}}$ zwei K.-Kreise im R_2 , $\mathfrak{x}(\sigma)$ eine orientierte lineare Konkruenzenschar und

$$\mathfrak{b}, \bar{\mathfrak{b}}$$

resp. die Kongruenzen \mathfrak{x} und die benachbarte Kongruenz $\mathfrak{x} + \dot{\mathfrak{x}} d\sigma$ berühren, so folgt

$$(5) \quad \begin{cases} \mathfrak{b}\mathfrak{b} = \mathfrak{b}\mathfrak{x} = \mathfrak{b}\mathfrak{x}' = 0, \\ \bar{\mathfrak{b}}\bar{\mathfrak{b}} = \bar{\mathfrak{b}}\mathfrak{x} = \bar{\mathfrak{b}}\mathfrak{x}' = 0, \end{cases}$$

wo

$$-\frac{d\xi}{d\sigma} = \xi'.$$

Wir leiten her eine neue Kongruenz η so wie

$$\eta\xi = \eta\xi' = \eta b = \eta\bar{b} = 0, \eta\eta = 1.$$

Wir können jetzt folgende Tabelle skalarer Produkte zwischen ξ, ξ', η, b und \bar{b} setzen.

(6)

	ξ	ξ'	η	b	\bar{b}
ξ	1	0	0	0	0
ξ'	0	1	0	0	0
η	0	0	1	0	0
b	0	0	0	0	1
\bar{b}	0	0	0	1	0

wo

$$|\xi, \xi', \eta, b, \bar{b}|^2 = 1.$$

Man kann daher setzen :

$$(7) \quad \begin{cases} \xi' = -\xi + a\eta + c\bar{b} + \bar{c}b, \\ \eta' = -a\xi' + d\eta + \bar{d}\bar{b}, \\ b' = -c\xi' - d\eta + e\bar{b}, \\ \bar{b}' = -\bar{c}\xi' - \bar{d}\eta - eb, \end{cases}$$

wo $-a, c, \bar{c}, d, \bar{d}, e$ skalare Grössen sind.

Man berücksichtige hier die folgende Tabelle skalarer Produkte :

	ξ	ξ'	η	η'	$\bar{\eta}$
ξ''	-1	0	a	c	\bar{c}
η''	0	$-a$	0	d	\bar{d}
η'	0	$-c$	$-d$	0	e
$\bar{\eta}'$	0	$-\bar{c}$	$-\bar{d}$	$-e$	0

Mit gleichen Methoden kann man die Formeln herleiten:⁽¹⁾

$$\xi' = -\xi + a\eta + b\zeta + cu + \bar{c}\bar{u},$$

$$\eta' = -a\xi' + f\zeta + ku + m\bar{u},$$

$$\zeta' = -b\xi' - f\eta + gu + e\bar{u},$$

$$u' = -c\xi' - k\eta - k\zeta + h\bar{u},$$

$$\bar{u}' = -\bar{c}\xi' - m\eta - e\zeta - hu,$$

wo

$$(\xi\xi')_e = (\eta\eta')_e = (\zeta\zeta')_e = 1, \quad (\xi\xi'')_e = 1,$$

$$(uu')_e = (\bar{u}\bar{u}')_e = 0, \quad (u\bar{u}')_e = 1,$$

$$(\xi\eta')_e = (\xi\zeta')_e = (\xi u')_e = (\xi\bar{u}')_e = 0,$$

$$(\xi'\eta)_e = (\xi'\zeta)_e = (\xi' u)_e = (\xi' \bar{u})_e = 0,$$

$$(\eta\zeta')_e = (\eta u')_e = (\eta\bar{u}')_e = 0, \quad (u\zeta')_e = (\bar{u}\zeta')_e = 0,$$

$$\frac{d\xi}{d\sigma} = \xi', \quad d\sigma = \sqrt{\frac{d\xi}{dt} \frac{d\bar{\xi}}{dt}} dt,$$

$a, b, c, \bar{c}, f, g, m, e, h, k$ skalare Grössen sind, weil

(1) Vergl. THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 140.

	ξ	ξ'	η	ζ	u	\bar{u}
ξ	1	0	0	0	0	0
ξ'	0	1	0	0	0	0
η	0	0	1	0	0	0
ζ	0	0	0	1	0	0
u	0	0	0	0	0	1
\bar{u}	0	0	0	0	1	0

	ξ	ξ'	η	ζ	u	\bar{u}
ξ''	-1	0	a	b	c	\bar{c}
η'	0	$-a$	0	f	k	m
ζ'	0	$-b$	$-f$	0	g	e
u'	0	$-c$	$-k$	$-g$	0	h
\bar{u}'	0	$-\bar{c}$	$-m$	$-e$	$-h$	0

Wir setzen

$$\langle \xi \eta \rangle = -x_0 y_0 + x_0 y_0 + x_1 y_1 + x_2 y_2 + x_3 y_3 + x_4 y_4 - x_5 y_5$$

$$(1) (\xi \xi)_0 = 0 (\langle \xi \xi \rangle_0 = 0), \quad (2) (\eta \xi)_0 = 0 (\langle \eta \eta \rangle_0 = 0)$$

bedeuten zwei K.-Kugeln.

Wenn sich zwei K.-Kugeln nicht berühren, so kann man setzen

$$(3) \quad \langle \xi \eta \rangle_e = 1,$$

wo u, v die Parameter bedeuten.

Nun kann man

$$(4) \quad \begin{cases} \sum dx_i dy_i = G_{ij} du^i dv^j, \\ \sum (dx_i)^2 = g_{ij} du^i du^j, \\ \sum (dy_i)^2 = \bar{g}_{ij} dv^i dv^j \end{cases}$$

nehmen.

Es ist so zu untersuchen wie in meiner Arbeit.⁽¹⁾

(8) Über Minimallinien

(1)

Hier betrachten wir den LAUERRESCHEN Winkel in der Kreisfläche. Die Gleichung von Minimallinien ist

$$(\theta_i \theta_t) dt^2 + 2(\theta_i \theta_\tau) dt d\tau + (\theta_\tau \theta_\tau) d\tau^2 = 0.$$

Bezeichnet man die Wurzel von

$$(\theta_i \theta_t) + 2k(\theta_i \theta_\tau) + k^2(\theta_\tau \theta_\tau) = 0$$

mit k_1, k_2 und k'_1, k'_2 , dann besteht

$$\frac{1+I}{i(1-I)} = \frac{(\theta_i \theta_t) dt d\tau + (\theta_i \theta_\tau) (\partial \tau dt + \partial t d\tau) + (\theta_\tau \theta_\tau) d\tau d\tau}{\sqrt{(\theta_i \theta_t)(\theta_\tau \theta_\tau) - (\theta_i \theta_\tau)^2} (d\tau dt - \partial \tau dt)},$$

wo

$$I = \frac{k_1 - k'_1}{k_2 - k'_1} : \frac{k_1 - k'_2}{k_2 - k'_2},$$

$$\omega = \frac{1}{2i} \log I \quad (i = \sqrt{-1}).$$

ω ist der LAUERRESCHER Winkel in unserer Kreisfläche.

(1) NAKAJIMA, S.: Differentialgeometrie der Kreisscharen, (X), (XI), (XII), Tôhoku Math. Journ. Vol. 34 (1931) p. 191.

(2)

Wenn unsere Kreisfläche eine geradlinige Fläche ist, so folgt

$$r = \frac{(\theta_i \theta_i) r_1 du^2 + (\theta_\tau \theta_\tau) r_2 dv^2}{(\theta_i \theta_i) du^2 + (\theta_\tau \theta_\tau) dv^2},$$

wo r_1, r_2 Maximum- und Minimumwerte von r sind. r bedeutet den Abstand von der Direktrix zu dem Punkt auf (u, v) -Gerade, die der Schnittpunkt mit gemeinsamer Normale zwischen zwei Geraden (u, v) und $(u+du, v+dv)$ ist,

Wenn λ, μ, ν den Dichtungskosinus von gemeinsamer Normale zwischen (u, v) und $(u+du, v+dv)$ bedeuten, so folgt

$$\begin{aligned} \lambda &= \frac{(\theta_i \theta_i) \frac{\partial X}{\partial v} - (\theta_\tau \theta_\tau) \frac{\partial X}{\partial u} dv}{\sqrt{(\theta_i \theta_i)(\theta_\tau \theta_\tau)} d\sigma}, \\ \mu &= \frac{(\theta_i \theta_i) \frac{\partial Y}{\partial v} du - (\theta_\tau \theta_\tau) \frac{\partial Y}{\partial u} dv}{\sqrt{(\theta_i \theta_i)(\theta_\tau \theta_\tau)} d\sigma}, \\ \nu &= \frac{(\theta_i \theta_i) \frac{\partial Z}{\partial v} du - (\theta_\tau \theta_\tau) \frac{\partial Z}{\partial u} dv}{\sqrt{(\theta_i \theta_i)(\theta_\tau \theta_\tau)} p\sigma}, \end{aligned}$$

wo X, Y, Z der Dichtungskosinus von Normale und $d\sigma$ der Winkel zwischen zwei Geraden (u, v) und $(u+du, v+dv)$ ist.

Betrachten wir Minimalflächen der Minimallinien als Parameter, so entsteht

$$(\theta_i \theta_i) = 0, \quad (\theta_\tau \theta_\tau) = 0.$$

(3)

Die Gleichung der Minimallinien auf Kreisflächen ist mit

$$(1) \quad (\theta_i \theta_i) dt^2 + 2(\theta_i \theta_\tau) dt d\tau + (\theta_\tau \theta_\tau) d\tau^2 = 0$$

gegeben.

Aus (1) folgt

$$(2) \quad \begin{cases} (\theta_i \theta_t) dt + [(\theta_i \theta_t)] + iV] d\tau = 0, \\ (\theta_i \theta_t) dt - [(\theta_i \theta_t)] iV] d\tau = 0, \quad (i = \sqrt{-1}), \end{cases}$$

wo

$$V^2 = (\theta_i \theta_t)(\theta_\tau \theta_\tau) - (\theta_i \theta_\tau)^2.$$

Nach Integration (2) ergibt sich:

$$(3) \quad \begin{cases} u = \phi(t, \tau) = \text{const.}, \\ v = \psi(t, \tau) = \text{const.}, \end{cases}$$

wo v die konjugierte Funktion von u ist.

So entsteht

$$(4) \quad \begin{cases} du = \mu[(\theta_i \theta_t) dt + \{(\theta_i \theta_\tau) + iV\} d\tau], \\ dv = \nu[(\theta_i \theta_t) dt + \{(\theta_i \theta_\tau) - iV\} d\tau], \end{cases}$$

wo μ, ν die konjugierten Grössen sind.

Aus

$$(5) \quad (\theta_i \theta_t) dt^2 + 2(\theta_i \theta_\tau) dt d\tau + (\theta_\tau \theta_\tau) d\tau^2 = 0$$

folgt also:

$$(6) \quad du dv = 0,$$

wo

$$(7) \quad \mu \nu (\theta_i \theta_t) \neq 0$$

ist.

Die Normalrichtung δn der Kurve

$$(8) \quad \phi(t, \tau) = \text{const}$$

auf einer Kreisfläche ist mit

$$(9) \quad \begin{cases} \frac{\delta t}{(\theta_\tau \theta_\tau) \phi_1 - (\theta_i \theta_\tau) \phi_2} = - \frac{\delta \tau}{(\theta_i \theta_t) \phi_2 - (\theta_i \theta_\tau) \phi_1} \\ = \frac{\partial u}{V_1 (\theta_i \theta_t) \phi_2^2 - 2(\theta_i \theta_\tau) \phi_1 \phi_2 + (\theta_\tau \theta_\tau) \phi_1^2}^{\frac{1}{2}} \end{cases}$$

gegeben.⁽¹⁾

Betrachten wir zwei Richtungen

$$(10) \quad \theta dt^2 + 2\phi dt d\tau + \psi d\tau^2 = 0$$

auf einer Kreisfläche und einer Richtung δt , $\delta \tau$, so folgt

$$(11) \quad \delta s^2 \cos \alpha \cos \beta = J/I,$$

wo α , β zwei Winkel zwischen $(\delta t, \delta \tau)$ und (10) bedeuten, δs das Bogenelement,⁽²⁾ und es sich verhält:

$$\begin{aligned} J &= [(\theta, \theta_t) \delta t + (\theta, \theta_\tau) \delta \tau]^2 \psi - \\ &\quad - 2\{(\theta, \theta_t) \delta t + (\theta, \theta_\tau) \delta \tau\} \{(\theta_t, \theta_\tau) \delta t + (\theta_\tau, \theta_\tau) \delta \tau\} \\ &\quad + \{(\theta_t, \theta_\tau) \delta t + (\theta_\tau, \theta_\tau) \delta \tau\}^2 \theta, \\ I &= \{(\theta_t, \theta_t) \psi - 2(\theta_t, \theta_\tau) \phi + (\theta_\tau, \theta_\tau) \theta\}^2 - \\ &\quad - 4\{(\theta_t, \theta_\tau) (\theta_\tau, \theta_\tau) - (\theta_t, \theta_\tau)\} (\theta \psi - \phi^2)^{\frac{1}{2}} \end{aligned}$$

(4)

Betrachten wir zwei Kreisflächen S und \bar{S} , so folgt

$$ds^2 = \frac{1}{\lambda} [(\theta_t, \theta_t) dt^2 + 2(\theta_t, \theta_\tau) dt d\tau + (\theta_\tau, \theta_\tau) d\tau^2],$$

$$d\bar{s}^2 = \frac{1}{\bar{\lambda}} [(\bar{\theta}_t, \bar{\theta}_t) dt^2 + 2(\bar{\theta}_t, \bar{\theta}_\tau) dt d\tau + (\bar{\theta}_\tau, \bar{\theta}_\tau) d\tau^2],$$

wo ds , $d\bar{s}$ die Bogenelemente von S und \bar{S} sind,

Wenn zwei geodetische Torsionen $\frac{1}{T}$ und $\frac{1}{\bar{T}}$ von S und \bar{S}

gleich und S , \bar{S} konformal sind, so folgt⁽³⁾

(1) FORSYTH, A. R.: Lectures on the Differential Geometry of Curves and Surfaces, Cambridge (1920) p. 159.

(2) FORSYTH, A. R.: op. cit.

(3) Vergl. OGURA, K.: Note on the Representation of Surfaces, Tôhoku Math. Journ. 10 (1916) p. 88.

$$\begin{aligned}
& [(\theta_i \theta_\tau \backslash \bar{q}_{11} - (\theta_i \theta_i \backslash \bar{q}_{12})][(\theta_\tau \theta_\tau \backslash \bar{q}_{12} - (\theta_i \theta_\tau \backslash \bar{q}_{22})] - [(\theta_\tau \theta_\tau \backslash \bar{q}_{11} - (\theta_i \theta_i \backslash \bar{q}_{22})]^2 \\
& = k^2 \{[(\theta_i \theta_\tau) q_{11} - (\theta_i \theta_i) q_{12}][(\theta_\tau \theta_\tau) q_{12} - (\theta_i \theta_\tau \backslash q_{22})] - [(\theta_i \theta_\tau \backslash q_{11} - (\theta_i \theta_i \backslash q_{22})]^2\}
\end{aligned}$$

(9) Kreis-und Kugelbüschel

(1)

Wir betrachten einen Kreisbüschel

$$(1) \quad \mathfrak{z} = \mathfrak{x} + i\mathfrak{y}, \quad \sqrt{-1} = i$$

im R_2 , wo \mathfrak{x} , \mathfrak{y} zwei Kreise im R_2 sind.

Wenn \mathfrak{z} ein Punkt ist, dann folgt aus (I)

$$0 = (\mathfrak{x}\mathfrak{x}) - (\mathfrak{y}\mathfrak{y}) + 2i(\mathfrak{x}\mathfrak{y})$$

d. h.

$$\begin{aligned}
(2) \quad & (\mathfrak{x}\mathfrak{x}) = (\mathfrak{y}\mathfrak{y}), \\
& (\mathfrak{x}\mathfrak{y}) = 0.
\end{aligned}$$

Aus (2) entsteht der

Satz: Wenn \mathfrak{z} in (1) ein Punkt ist, dann sind die Kreise \mathfrak{x} und \mathfrak{y} zueinander nicht senkrecht.

Wenn

$$\mathfrak{z} = \mathfrak{x} + i\mathfrak{y}, \quad i = \sqrt{-1}$$

und

$$\bar{\mathfrak{z}} = \bar{\mathfrak{x}} - i\bar{\mathfrak{y}}$$

zueinander senkrecht sind, dann folgt

$$0 = (\mathfrak{x}\bar{\mathfrak{x}}) + i(\mathfrak{x}\bar{\mathfrak{y}} - \mathfrak{y}\bar{\mathfrak{x}}) + (\mathfrak{y}\bar{\mathfrak{y}}),$$

d. h.

$$\begin{cases} (\mathfrak{x}\mathfrak{x}) + (\mathfrak{y}\bar{\mathfrak{y}}) = 0, \\ (\mathfrak{x}\bar{\mathfrak{x}}) = (\mathfrak{x}\bar{\mathfrak{y}}), \end{cases}$$

so folgt der

Satz: Der Winkel zwischen \bar{x} und y ist gleich dem Winkel zwischen x und \bar{y} .

Nun beweise ich den folgenden Satz.

Satz: Wenn x, y, z die zueinander senkrechten Kreise im R_2 sind, so kann

$$x + iy + \lambda z, \quad i = \sqrt{-1}$$

kein Punkt sein, wo λ ein Parameter ist.

Beweis: Sei

$$p = x + iy + \lambda z$$

ein Punkt, dann folgt

$$0 = (xx) - (yy) + \lambda^2 (zz) + 2i(xy) + 2\lambda(zz) + 2i\lambda(yz),$$

$$\text{d. h.} \quad 0 = \lambda^2 \quad \text{oder} \quad \lambda = 0,$$

so ergibt sich

$$p = x + iy,$$

d. h. ein Kreis durch die Schnittpunkte von x und y .

(2)

Wir betrachten den Kreisbüschel

$$(1) \quad \mathfrak{A}(u^1, u^2, u^3) = a(u^1, u^2, u^3) + \lambda \bar{a}(u^1, u^2, u^3),$$

im R_4 , wo u^i drei Parameter, λ eine Konstante und a, \bar{a} zwei Kugeln im R_4 sind,

Die quadratische Differentialform von \mathfrak{A} ist

$$(2) \quad d\mathfrak{A} = \sum_{i,k=1}^3 G_{ik} du^i du^k,$$

wo

$$(3) \quad G_{ik} = g_{ik} + \lambda \bar{g}_{ik}, \quad da = \sum_{i,k=1}^3 g_{ik} du^i du^k, \quad \text{u. s. w..}$$

Die Determinante musz sein :

$$(4) \quad D = \begin{vmatrix} G_{11} & G_{12} & G_{13} \\ G_{12} & G_{22} & G_{23} \\ G_{13} & G_{23} & G_{33} \end{vmatrix}.$$

Betrachten wir den Kreisbüschel

$$\mathfrak{g} = \rho_a \mathfrak{U}^a, \quad [a = I, II],$$

so folgt

$$(\mathfrak{g}\mathfrak{g}) = \rho_a \rho_b A^{ab},$$

wo

$$A^{ab} = (\mathfrak{U}^a \mathfrak{U}^b), \quad A^{ab} | = A,$$

$$A_{11} = \frac{1}{A} (A^{22} A^{31} - A^{21} A^{32}), \quad A_{12} = -\frac{1}{A} (A^{11} A^{21} - A^{21} A^{31}),$$

$$A_{13} = -\frac{1}{A} (A^{21} A^{32} - A^{11} A^{22}), \quad A_{22} = \frac{1}{A} (A^{11} A^{31} - A^{31} A^{11})$$

$$A_{23} = \frac{1}{A} (A^{11} A^{32} - A^{12} A^{31}), \quad A_{33} = \frac{1}{A} (A^{11} A^{22} - A^{12} A^{21})$$

sind.

Wollen wir nun einen eigentlichen reellen Punkt haben, so müssen wir $A > 0$ voraussetzen.

Es gilt

$$A^{ab} A_{b\gamma} = \begin{cases} 1 & \text{für } a = \gamma \\ 0 & \text{,, } a \neq \gamma \end{cases}$$

$$-\frac{1}{2} A^{ab} A_{ab} = 1.$$

$$\mathfrak{U}^a \mathfrak{U}^a = 0 \quad [a = I, II, III]$$

bedeutet, daß die Punkte

$$\mathfrak{U}^a \quad [a = I, II, III], \quad (\mathfrak{U}\mathfrak{U}) = 1$$

auf einer Kugel \mathfrak{U} sind.⁽¹⁾

(1) Vergl. NAKAJIMA, S.: Differentialgeometrie der Hyperboloidscharen, Tôhoku Math. Journ. 31 (1929) p. 247.

(3)

Vier einem Büschel angehörige Kreise⁽¹⁾

$$\mathfrak{K}_{(\sigma)} = \alpha_{(\sigma)}\mathfrak{A} + \beta_{(\sigma)}\mathfrak{B} \quad (\sigma=1, 2, 3, 4)$$

im R_2 haben eine Invariante, das Doppelverhältnis

$$D = \frac{(\alpha_1\beta_2 - \alpha_2\beta_1)(\alpha_3\beta_4 - \alpha_4\beta_3)}{(\alpha_1\beta_4 - \alpha_4\beta_1)(\alpha_2\beta_3 - \alpha_3\beta_2)},$$

wobei

$$(\mathfrak{K}\mathfrak{K})_1 = 1, (\mathfrak{A}\mathfrak{A}) = 1, (\mathfrak{B}\mathfrak{B}) = 0.$$

Die entsprechende Invariante von vier Kreisen im R_2 eines Büschels werden wir als das Winkelverhältnis bezeichnen.

Den Ausdruck

$$\varphi = \frac{i}{2} \log D$$

nennen wir das logarithmische Doppelverhältnis,

Für φ gilt die Formel

$$\operatorname{ctg} \varphi = i \frac{1+D}{1-D}.$$

Die vier Elemente des Büschels haben das harmonische Doppelverhältnis, wenn

$$D = -1, \quad \varphi = -\frac{\pi}{2}$$

ist.

(4)

Betrachten wir den Kreisbüschel

$$(1) \quad \hat{\xi} = \alpha\xi + \beta\eta$$

(1) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925 p. 123.

im R_2 , wo α, β zwei veränderliche skalare Grössen, ξ, η zwei feste Kreise im R_2 sind.

Die Bedingung dafür, dass $\hat{\xi}$ die sich konsekutiven Kreise berühren, ist⁽¹⁾ die :

$$(2) \quad (\dot{\hat{\xi}}\dot{\hat{\xi}})=0.$$

In unserem Falle bestehen

$$(3) \quad (\dot{\hat{\xi}}\dot{\hat{\xi}}) \neq 0,$$

weil

$$(4) \quad (\dot{\hat{\xi}}\dot{\hat{\xi}}) = \alpha^2(\dot{\xi}\dot{\xi}) + \beta^2(\dot{\eta}\dot{\eta})$$

ist, so entsteht der

Satz: *Unsere Kreisbüschel $\hat{\xi}$ in (1) können die nicht sich konsekutiven Kreise berühren.*

Betrachten wir den Fall, dass zwei Kreise ξ und η in (1) veränderlich sind, so folgt aus (1), dass *nur in dem Falle ξ und η die sich konsekutiven Kreise berühren und $\hat{\xi}$ auch sich berühren.*

(5)

Ist $\hat{\xi}$ ein fester Kreis und ξ ein nicht auf ihm gelegener Kreis im R_2 , so ist⁽²⁾

$$(1) \quad \eta = 2(\xi\hat{\xi})\hat{\xi} - \xi$$

der zu ξ bezüglich des Kreises $\hat{\xi}$ inverse Kreis.

Wenn η und ξ sich in einem Punkt ξ berühren, so folgt⁽³⁾

$$(2) \quad \xi = \xi - (\xi, 2(\xi\hat{\xi})\hat{\xi} - \xi) \cdot \overline{2(\xi\hat{\xi})\hat{\xi} - \xi},$$

wo

$$(3) \quad (\xi, 2(\xi\hat{\xi})\hat{\xi} - \xi)^2 = 1,$$

(1) THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamburgischen Univ. Bd. IV. S. 126.

(2) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 123.

(3) THOMSEN, G.: op. cit.

so folgt

$$(4) \quad \begin{cases} \xi = 2\delta - 2(\delta\hat{\xi})\hat{\xi}, \\ \xi = 2(\delta\hat{\xi})\hat{\xi}. \end{cases}$$

Aus (4) ergibt sich

$$(\xi\hat{\xi}) = 0.$$

So entsteht der

Satz: In unserem Falle musz der Punkt ξ auf $\hat{\xi}$ liegen.

Aus $(\dot{\eta}\dot{\eta}) = 0$ in (1) kommt

$$(\dot{\delta}\dot{\delta}) = 0,$$

so folgt der

Satz: Wenn ein Kreis η im R_2 die sich konsekutiven Kreise berührt, so berührt der inverse Kreis δ die in Bezug auf den Kreis $\hat{\xi}$ auch dem η konsekutiven Kreise.

(6)

Betrachten wir die Kugelscharen

$$(1) \quad \gamma = x\hat{\xi}^* + \xi\gamma^*,$$

im R_n , wo ξ, γ die Kugeln im R_n sind und

$$(2) \quad (\gamma\gamma) = x^2(\hat{\xi}^*\hat{\xi}^*) + 2xy(\hat{\xi}^*\gamma^*) + y^2(\hat{\xi}^*\gamma^*) - 1 = 0$$

besteht, so ergibt sich

$$(3) \quad \cos\varphi = x(\hat{\xi}^*\hat{\xi}) + y(\gamma^*\hat{\xi}),$$

wo φ den Winkel zwischen zwei Kugeln γ und $\hat{\xi}$ im R_n bedeutet und x, y skalare Grössen sind.

Nun setzen wir

$$g(x, y) = x^2(\hat{\xi}^*\hat{\xi}^*) + 2xy(\hat{\xi}^*\gamma^*) + y^2(\hat{\xi}^*\gamma^*) - 1,$$

$$f(x, y) = x(\hat{\xi}^*\hat{\xi}) + y(\gamma^*\hat{\xi}),$$

$$p = -\frac{\partial f}{\partial x} = (\xi^* \delta),$$

$$q = -\frac{\partial f}{\partial y} = (\gamma^* \delta),$$

$$p_1 = -\frac{\partial g}{\partial x} = 2[x(\xi^* \xi^*) + y(\xi^* \gamma^*)],$$

$$q_1 = -\frac{\partial g}{\partial y} = 2[x(\xi^* \gamma^*) + y(\xi^* \gamma^*)],$$

dann erfolgt sich aus

$$j = pq_1 - qp_1 = 0$$

$$j = 2\{(\xi^* \delta)[x(\xi^* \gamma^*) + y(\xi^* \gamma^*)] - (\gamma^* \delta)[x(\xi^* \xi^*) + y(\xi^* \gamma^*)]\}$$

so folgt der

Satz: Die Anzahl des Maximums und Minimums von $\cos \varphi$ ist zwei.

(7)

Nach MUKHOPADHYAYA⁽¹⁾ nennt man einen Punkt einer Kurve, in welchem der Krümmungskreis wenigstens vier sich konsekutive Punkte enthält, einen zyklischen Punkt.

Im folgenden wollen wir den zyklischen Punkt bestimmen.

Sind b, \bar{b} die beiden Schnittpunkte einer Kreisschar $\xi(\sigma)$ im R_2 , so gilt⁽²⁾

$$(1) \quad \begin{cases} (bb) = (b\xi) = (b\xi') = (b\xi'') = 0, \\ (\bar{b}\bar{b}) = (\bar{b}\xi) = (\bar{b}\xi') = (\bar{b}\xi'') = 0. \end{cases}$$

Dann haben wir die folgende Tabelle der skalaren Produkte :

-
- (1) MUKHOPADHYAYA, S.: New Method in the Geo. of a Plane, Arc, Bull. Calcutta Math. Sc., Vol. I (1909).
- (2) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Sem. der Hamb. Univ. Univ. IV Bd. (1925 S. 126.

(2)

—	ξ	ξ'	ξ''	\mathfrak{b}	$\bar{\mathfrak{b}}$
ξ	1	0	-1	0	0
ξ'	0	1	0	0	0
ξ''	-1	0	d	0	0
\mathfrak{b}	0	0	0	0	1
$\bar{\mathfrak{b}}$	0	0	0	1	0

 wo $(\xi''\xi'')=d$ ist.

(8)

Setzen wir

$$\xi_1 = \sigma \frac{X}{R}; \quad \xi_2 = \sigma \frac{Y}{R}; \quad \xi_3 = \sigma \frac{X^2 + Y^2 - R^2 - 1}{2R};$$

$$\xi_4 = \sigma \frac{X^2 + Y^2 - R^2 + 1}{2R};$$

$$\tau_1 = \rho \frac{x}{R}; \quad \tau_2 = \rho \frac{y}{R}; \quad \tau_3 = \rho \frac{x^2 + y^2 - R^2 - 1}{2R};$$

$$\xi_4 = \rho \frac{x^2 + y^2 - R^2 + 1}{2R}$$

in

$$(\xi_3) = 0 \quad \text{ein,}$$

so folgt

$$(X-x)^2 + (Y-y)^2 = 0,$$

Also erhalten wir den

Satz: Wenn sich zwei Kreise ξ, η im R_2 gleichsinnig berühren, so müssen sie miteinander zusammenfallen, wo ξ, η denselben Radius haben.

(9)

Nach Mühlbach⁽¹⁾ besteht

$$(1) \quad d\sigma^2 = \sqrt{\left(-\frac{1}{\rho\tau}\right)^2 + \left(\frac{\dot{\sigma}}{\rho^2}\right)^2} dt^2.$$

Nach Bonnet folgt

$$(2) \quad \delta = -\frac{M_1 M_2}{12\rho\tau},$$

wo M_1, M_2 zwei benachbarte Punkte auf einer Raumkurve und δ der kleinste Abstand zwischen zwei Tangenten in M_1, M_2 ist.

Aus (1), (2) ergibt sich:

$$(3) \quad d\sigma^2 = \sqrt{\left(-\frac{12\delta}{M_1 M_2}\right)^2 + \left(\frac{\dot{\rho}}{\rho^2}\right)^2} dt^2.$$

(10)

$$(1) \quad \mathfrak{U}(u^1, u^2) + \varepsilon \mathfrak{U}(u^1, u^2)$$

bezeichnet ∞^2 Kreisscharen im R_3 , wo $\mathfrak{U}, \mathfrak{U}$ die Kugeln im R_3 , ε die Dualen Zahlen sind.

Setzen wir

$$(2) \quad A = \mathfrak{U} + \varepsilon \bar{\mathfrak{U}},$$

so folgt

$$(3) \quad dA^2 = G_{ik} du^i du^k,$$

wo

- (1) MUHLBACH, R.: Über Raumkurven in der Möbiusschen Geometrie, Sitzungsberichte der Heidelberger Akad. der Wiss. Jahrgang 1928, II Abhandlung. S. 5.

$$(4) \quad G_{ik} = A_{ik} A_{ik}$$

ist. Wir setzen noch

$$(5) \quad G_{ik} = \mathfrak{A}_{ik} + \varepsilon \bar{\mathfrak{A}}_{ik}.$$

Aus (2) folgt der

Satz: Um die Kreisscharen (I) zu bezeichnen, müssen \mathfrak{A} und $\bar{\mathfrak{A}}$ zueinander senkrecht sein.

(11)

Aus (28) in BLASCHKES Buch⁽¹⁾ kann man wissen, dass, wenn

$$(1) \quad \begin{cases} (\mathfrak{z} \mathfrak{v}) = 0, \\ (\mathfrak{z} \bar{\mathfrak{v}}) = 0, \\ (\mathfrak{z} \dot{\mathfrak{z}}) = 0, \\ (\mathfrak{v}' \mathfrak{z}) = 0 \end{cases}$$

bestehen, auch die Gleichungen gelten :

$$(2) \quad \begin{cases} (\mathfrak{z} d\dot{\mathfrak{z}}) = 0 \\ (\mathfrak{z} d^2\dot{\mathfrak{z}}) = 0 \end{cases}$$

d. h.

$$\begin{aligned} (\mathfrak{z} \dot{\mathfrak{z}}) &= 0 \\ (\mathfrak{z} \{\dot{\mathfrak{z}} + d\dot{\mathfrak{z}}\}) &= 0, \\ (\mathfrak{z} \{\dot{\mathfrak{z}} + d\dot{\mathfrak{z}} + \frac{1}{2} d^2\dot{\mathfrak{z}}\}) &= 0 \end{aligned}$$

bestehen.

Also folgt der

Satz: Wenn ein Kreis \mathfrak{z} durch die Punkte \mathfrak{v} , $\bar{\mathfrak{v}}$ hingeht und zu der konsekutiven Bogenlänge $\mathfrak{v} + d\mathfrak{v}$ senkrecht ist, so ist \mathfrak{z} zu den

(1) BLASCHKE, W.: Vorlesungen über Differentialgeometrie III, Berlin (1929), S. 99.

konsekutiven Kreisen ξ , $d\xi$, $\xi + d\xi + \frac{1}{2}d^2\xi$ senkrecht. Umgekehrt ist \mathfrak{z} zu den konsekutiven Kreisen

$$\xi, d\xi, \xi + d\xi + \frac{1}{2}d^2\xi$$

senkrecht, ebenso ist \mathfrak{z} auch zu den konsekutiven Bogenelementen $\mathfrak{v} + d\mathfrak{v}$ senkrecht.

(12)

$$(1) \quad \mathfrak{v} = \xi - 2\eta, \text{ wo } (\mathfrak{v}\mathfrak{v}) = 0, (\xi\xi) = (\eta\eta) = 1.$$

Demnach erfolgt es, dass sich zwei Kreise ξ , η in \mathfrak{v} berühren.

Aus (1) und (30) in THOMSENS Arbeit⁽¹⁾ ergibt sich:

$$(2) \quad \begin{cases} \xi_{oo} = (c-1) \xi - \epsilon c \eta + c \bar{\xi} - \epsilon c \bar{\eta}, \\ (1+c)\xi_o = \epsilon \eta_o, \\ (1+\bar{c})\bar{\xi}_o = \epsilon \bar{\eta}_o. \end{cases}$$

(2) ist unsere Gleichung, wo $\epsilon = \pm 1$ ist.

Die elementare Tangente kann mittels eines Parameters r folgendermassen dargestellt werden:

$$\eta = \mathfrak{v} + r\mathfrak{v}_o$$

$$\text{d. h.} \quad \eta = \mathfrak{v} - c r \xi_o,$$

wo η die laufende Koordinate ist.

Die Gleichung der Schmiegebene der Raumkurve⁽²⁾ ist

$$(\eta - u, u', u') = 0,$$

$$\text{d. h.} \quad (\eta - u, d_o^2 \zeta, d_o^2 \zeta + d_o^2 \bar{\zeta}) = 0$$

(1) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 127.

(2) THOMSEN, G.: op. cit.

(13)

Wenn die Kreise $\xi(t)$ im R_2 sind, dann sind die Schmiegkreise⁽¹⁾ der Kurve $\xi(t)$.

Sind $\xi(t+dt)$ die benachbarten Kreise von $\xi(t)$, so folgt

$$\xi(t+dt) = \xi(t) + t d\xi(t) + \frac{t^2}{2} d^2\xi + \dots$$

Also ergibt sich:

$$\begin{aligned} (\eta(t), \xi(t+dt)) &= (\xi(t)\xi(t)) + t(\xi(t)d\xi(t)) + \\ &+ \frac{t^2}{2}(\xi(t)d^2\xi(t)) + \dots \end{aligned}$$

Wenn t klein ist, so ist der Winkel zwischen $\xi(t)$ und $\xi(t+dt)$ gleich

$$1 - \frac{t^2}{2}(d\xi(t)d\xi(t)) + \dots,$$

wo $(\xi(t), \xi(t)) = 1$ ist.

(14)

Wir betrachten zwei Geraden

$$\bar{h} = \rho_\alpha \xi^\alpha, \quad \bar{h} = \rho_\lambda \bar{\xi}^\lambda,$$

so folgt⁽²⁾

$$A^{\alpha\beta} \rho_\alpha \rho_\beta = 0, \quad \bar{A}^{\lambda\mu} \rho_\lambda \rho_\mu = 0.$$

Betrachten wir⁽³⁾

$$\bar{D}^{\alpha\lambda} \rho_\alpha \rho_\lambda.$$

Setzen wir

- (1) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 126.
- (2) THOMSEN, G.: Über konforme Geometrie, Abh. aus dem Math. Seminar der Hamb. Univ. Bd. IV (1926) S. 249.
- (3) THOMSEN, G.: op. cit.

$$f \equiv D^{\alpha\lambda} \rho_\alpha \rho_\lambda,$$

$$g_1 \equiv A^{\alpha\beta} \rho_\lambda \rho_\mu,$$

$$g_2 \equiv \bar{A}^{\lambda\mu} \rho_\lambda \rho_\mu,$$

dann folgt

$$\frac{\partial f}{\partial \rho_1} = \text{lineare Gleichung in } \rho_1, f, \rho_{II}, \rho_{III},$$

$$\frac{\partial f}{\partial \rho_2} = \quad \quad \quad ,$$

$$\frac{\partial f}{\partial \rho_3} = \quad \quad \quad ,$$

$$\frac{\partial g_1}{\partial \rho_1} = \quad \quad \quad ,$$

$$\frac{\partial g_1}{\partial \rho_2} = \quad \quad \quad ,$$

$$\frac{\partial g_1}{\partial \rho_3} = \quad \quad \quad ,$$

$$\frac{\partial g_2}{\partial \rho_1} = \quad \quad \quad ,$$

$$\frac{\partial g_2}{\partial \rho_2} = \quad \quad \quad ,$$

$$\frac{\partial g_2}{\partial \rho_3} = \quad \quad \quad .$$

Aus

$$\begin{vmatrix} \frac{\partial f}{\partial \rho_1} & \frac{\partial f}{\partial \rho_2} & \frac{\partial f}{\partial \rho_3} \\ \frac{\partial g_1}{\partial \rho_1} & \frac{\partial g_1}{\partial \rho_2} & \frac{\partial g_1}{\partial \rho_3} \\ \frac{\partial g_2}{\partial \rho_1} & \frac{\partial g_2}{\partial \rho_2} & \frac{\partial g_2}{\partial \rho_3} \end{vmatrix}$$

folgt dann

$j = (\text{Gleichung des dritten Grades in } \rho_1, \rho_2, \rho_3).$

Die Stelle fürs Maximum und Minimum von $j=0$ auf $g_1=0$, $g_2=0$ muss auf der Fläche $j=0$ sein, also folgt der

Satz: Die Anzahl des Maximums und Minimums j ist $3 \cdot \infty^1$.

(15)

Nehmen wir zwei zueinander senkrechte Kreischaren $\eta(t)$ und $\zeta(t)$ im R_2 , dann bezeichnet

$$(1) \quad v = l\eta + m\zeta$$

einen Kreisbüschel durch den Schnittpunkt von η und ζ , wo t ein Parameter ist.

Wenn (1) die Punkte bezeichnet, so folgt

$$(vv) = (l\eta + m\zeta, l\eta + m\zeta) = 0,$$

$$\text{d. h.} \quad l^2 + m^2 = 0,$$

wo l, m zwei Parameter sind.

Es gilt daher die Beziehung:

$$(2) \quad \begin{aligned} v(t) &= \frac{1}{\mu} (\zeta(t) + i\eta(t)), \\ \bar{v}(t) &= -\frac{1}{\mu} (\zeta(t) - i\eta(t)), \end{aligned}$$

wo μ und $\bar{\mu}$ beliebige Funktionen von t sind.

Nun betrachten wir ein System von den Schmiegunskreisen $\xi(t)$, die v und \bar{v} umhüllen. Es ergibt sich:

$$\xi_{oo} = -\xi + c\bar{v} + \bar{c}v,$$

$$\bar{v}_o \equiv \left\{ -\frac{1}{\mu} (\zeta - i\eta) \right\}_o = -\dot{c} \xi_o,$$

$$v_o \equiv \left\{ \frac{1}{\mu} (\zeta + i\eta) \right\}_o = -\dot{\bar{c}} \xi_o,$$

$$\xi_0 = \frac{d\xi}{d\sigma}, \quad \text{u. s. w.,}$$

$$d\sigma = (\xi, \xi_t) dt,$$

$$\xi_0 = \xi_t \frac{dt}{d\sigma} = \xi_t / (\xi, \xi_t),$$

$$\frac{d\sigma}{dt} = (\xi, \xi_t) = 1 / \rho,$$

$$c^2 = \rho^2,$$

wo ρ konforme Krümmung, $d\sigma$ Kontingenzwinkel bedeuten.

(16)

Nach MÜHLBACH findet statt :

$$(1) \quad d\sigma = \sqrt{\left(\frac{1}{\rho\tau}\right)^2 + \left(\frac{\dot{\rho}}{\rho}\right)^2} dt,$$

wo $\frac{1}{\tau}$ = eine Windung und ρ = ein Krümmungsradius ist.

Für den Radius R der Schmiegunskugel ergibt sich :⁽¹⁾

$$(2) \quad R^2 = \rho^2 + \tau^2 \dot{\rho}^2.$$

Aus (1) und (2) folgt

$$(3) \quad d\sigma^2 = \frac{1}{\rho^2} \sqrt{-\frac{\dot{\rho} R^2}{R^2 - \rho^2}} dt.$$

Wenn zwischen der Krümmung und der Torsion eine lineare Gleichung mit konstanten Koeffizienten der Form

$$(4) \quad \frac{A}{\tau} + \frac{B}{\rho} = c$$

besteht, nennt man solche Kurven Betrandsche Kurven.⁽²⁾

(1) KOMMERELL, V. und KOMMERELL, K.: Theorie der Raumkurven und krummen Flächen I, (1931) S. 35.

(2) KOMMERELL, V. und KOMMERELL, K.: l. c.

Für (4) ergibt sich

$$(5) \quad d\sigma^2 = \sqrt{\left(\frac{1}{a\rho + \beta\tau}\right)^2 + \left(\frac{\dot{\rho}}{\rho}\right)^2} dt^2,$$

wo a, β zwei Konstanten sind.

Ist die gegebene Kurve eben, so folgt⁽¹⁾

$$(6) \quad \frac{1}{\tau} = 0.$$

Aus (1), (6) ergibt sich

$$d\sigma^2 = \dot{\rho}^2 / \rho^2 dt^2,$$

d. h. (7) $\rho = e^{\sigma}.$

Für allgemeine Zylinder⁽²⁾

$$(8) \quad \frac{\tau}{\rho} = \text{const},$$

erfolgt

$$(9) \quad d\sigma^2 = \frac{1}{\rho^2} \sqrt{\frac{1}{c^2} + \dot{\rho}^2} dt^2,$$

wo c eine Konstante ist.

Nehmen wir⁽³⁾

$$\frac{1}{\rho} = \frac{p}{q}, \quad \frac{1}{\tau} = \frac{q}{q},$$

so folgt

$$d\sigma^2 = \sqrt{\left(\frac{pq}{\dot{p}\dot{q}}\right)^2 + \left(\frac{\bar{q}\dot{p} - \dot{p}\bar{q}}{\dot{q}^2}\right)^2} dt^2.$$

(1) KOMMERELL, V. und KOMMERELL, K.: l. c.

(2) KOMMERELL, V. und KOMMERELL, K.: l. c.

(3) BLASCHKE, W.: Vorlesungen über Differentialgeometrie I, Berlin (1930) S. 273.

(17)

Satz: Wenn sich zwei Raumkurven im COMBESCURESchen Korrespondieren verhalten, so muss

$$\frac{\tau}{\tau_1} = \frac{\rho}{\rho_1}$$

sein, wo

$$(A) \quad d\sigma : dt = d\sigma_1 : dt_1,$$

Beweis: Setzen wir

$$(1) \quad \frac{\rho}{k} = \frac{\tau}{k} = 1$$

so folgt

$$(2) \quad \rho = k\rho_1, \quad \tau = k\tau_1.$$

Aus (2) und

$$(3) \quad d\sigma^2 = \sqrt[2]{\left(\frac{1}{\rho\tau}\right)^2 + \left(\frac{\dot{\rho}}{\rho^2}\right)^2} dt^2$$

ergibt sich

$$(4) \quad d\sigma^2 = \frac{1}{k} \sqrt[2]{\frac{1}{k^2} \left(\frac{1}{\rho_1\tau_1}\right)^2 + \left(\frac{\dot{\rho}_1}{\rho_1^2}\right)^2} dt.$$

Aus (4),

$$(5) \quad d\sigma_1^2 = \sqrt[2]{\left(\frac{1}{\rho_1\tau_1}\right)^2 + \left(\frac{\dot{\rho}_1}{\rho_1^2}\right)^2} dt_1,$$

und (A) entwickelt sich

$$k=1, \quad \text{w. z. b. w..}$$

Wenn die Raumkurven mit einer festen Richtung einen festen Winkel H haben, so folgt

$$\frac{\tau}{\rho} = \cot H.$$

Daraus ergibt sich

$$\begin{aligned} d\sigma^2 &= \sqrt{\left(\frac{1}{\rho^2}\right)^2 + \left(\frac{\dot{\rho}}{\rho^2}\right)^2} dt^2 \\ &= \frac{1}{\rho^2} \sqrt{\dot{\rho}^2 \tan^2 H + \rho^2} dt^2. \end{aligned}$$

(10) Dreifachorthogonalsysteme

Mit

$$\mathfrak{x} = \mathfrak{x}(u^1, u^2, u^3), \quad (\mathfrak{x}_3 = 0, u^1 = u, u^2 = v, v^3 = w)$$

bezeichnen wir den Flächenpunkt eines dreifachorthogonalen Flächensystems im konformen Raum derart, dass die Gleichungen

$$u = \text{const.}, \quad v = \text{const.}$$

und

$$w = \text{const.}$$

die drei Systeme von den zueinander senkrecht stehenden Flächen darstellen.

Dann ist das konforme Bogenelement durch

$$\begin{aligned} ds^2 &= d\mathfrak{x} d\mathfrak{x} = G_{hk} du^h du^k \\ &= H_h^2 du^h du^h, \quad (H_h > 0) \end{aligned}$$

gegeben, da

$$\mathfrak{x}_r \mathfrak{x}_{sr} = \mathfrak{x}_{sr} \mathfrak{x}_r = \mathfrak{x}_u \mathfrak{x}_r = 0$$

sein soll.

Es seien die Tangentenkugeln \mathfrak{z}^h im Punkte \mathfrak{x} an die Flächen

$$u^h = \text{const.}$$

durch folgende Gleichungen gegeben:⁽¹⁾

$$\mathfrak{z}^h = \mathfrak{z}^h(u, v, w), \quad (\mathfrak{z}^h \mathfrak{z}^h = 1), \quad h = 1, 2, 3,$$

(1) TAKASU, T.: Über dreifachorthogonale Systeme im konformen Raume, Monatshefte für Math. und Physik, 37, 1930, S. 13.

so folgt

$$H_a = \xi_a \dot{\xi}^a \quad \text{u. s. w..}$$

Sei T die kinematische Energie eines Masspunktes mit Einheit-mass, so folgt

$$T = \frac{1}{2} (H_1^2 \dot{u}_1^2 + H_2^2 \dot{u}_2^2 + H_3^2 \dot{u}_3^2),$$

wo der Punkt das Differenzieren nach der Zeit t bedeutet.

LAGRANGES Gleichung von Bewegung wird

$$\begin{aligned} \frac{d}{dt} (H_1^2 \dot{u}^2) - \frac{1}{2} \frac{\partial H_1^2}{\partial u_1} \dot{u}_1^2 &= -\frac{\partial U}{\partial u_1}, \\ -\frac{1}{2} \frac{\partial H_1^2}{\partial u_2} \dot{u}^2 &= \frac{\partial U}{\partial u_2}, \\ -\frac{1}{2} \frac{\partial H_1^2}{\partial u_3} \dot{u}_1^2 &= \frac{\partial U}{\partial u_3}, \end{aligned}$$

wo U eine Kraftfunktion ist und

$$u_2 = \text{const.},$$

$$u_3 = \text{const.}$$

mit einem Masspunkt geschrieben sind.

Nach Ogura folgt⁽¹⁾

$$(1) \quad -\frac{1}{2} \frac{\partial}{\partial u_1} (H_1^2 \dot{u}_1^2) = -\frac{\partial U}{\partial u_1}.$$

Aus (1) ergibt sich

$$\frac{1}{2} \frac{\partial}{\partial u_1} (\xi_1 \dot{\xi}^1 \dot{u}_1^2) = \frac{\partial U}{\partial u_1}.$$

I. Wenn

$$\frac{\partial H_1}{\partial u_2} \neq 0 \quad \text{und} \quad \frac{\partial H_1}{\partial u_3} \neq 0,$$

(1) OGURA, K.: Trajectories in the conservative field of force, II, Tôhoku Math. Journ. 8 (1915) p. 197.

30

$$(2) \quad (\xi_1 \xi')^{\frac{1}{2}} (U + h) = \phi(u_1),$$

wo ϕ eine beliebige positive Funktion von u_1 und h eine konstante totale Energie ist.

II. Wenn

$$\frac{\partial H_1}{\partial u_2} = 0 \quad \text{und} \quad \frac{\partial H_1}{\partial u_3} \neq 0,$$

so folgt (2) auch.

III. Wenn

$$\frac{\partial H_1}{\partial u_2} = 0 \quad \text{und} \quad \frac{\partial H_1}{\partial u_3} = 0$$

so erfolgt⁽¹⁾ auch (2).

(11) Beiträge zur Inversionsgeometrie und Laguerre-Geometrie

(1)

In dieser Note vergleiche ich die beiden Arbeiten von KUBOTA⁽¹⁾ mit THOMSENS⁽²⁾ Arbeit und setze dadurch die Fundamentalsätze über Inversionsgeometrie und LAGUERRE-Geometrie in eine etwas modifizierte Form.

Bezeichnet man nämlich die natürliche Gleichung der Ebenenkurve mit

$$\rho' = \rho'(t),$$

wo ρ den Krümmungsradius und t die Kurvenlänge bedeutet, dann bleibt

$$\frac{d\rho \, d\sigma}{\rho}$$

durch MÖBIUSSche Invalution ungeändert, während sich sein Vorzeichen durch die Inversion ändert.

(1) MATSUMURA, S.: Beiträge zur Geo. der Kreise und Kugeln (I, Mem. of the Fac. of Sci. and Agr. Taihoku Imp. Univ. Vol. 5 (1932) p. 124.

Das Gleiche gilt für

$$\begin{aligned}
 I = & \frac{1}{2} \left(\frac{\rho}{\frac{d\rho}{d\sigma}} - \frac{1}{\rho} \frac{d\rho}{d\sigma} \right) + \frac{1}{2} \frac{\frac{d^2\rho}{d\sigma^2} - \left(\frac{d\rho}{d\sigma} \right)^2}{\frac{d\rho}{d\sigma}} - \\
 & - \frac{\rho \frac{d^3\rho}{d\sigma^3} - 2 \left(\frac{d\rho}{d\sigma} \frac{d^2\rho}{d\sigma^2} - \rho \frac{d^2}{d\sigma^2} \frac{d^2\rho}{d\sigma^2} + 2 \left(\frac{d\rho}{d\sigma} \right)^2 \right)}{2 \left(\frac{d\rho}{d\sigma} \right)^2} + \\
 & + \frac{5}{8} \frac{\rho \left(\frac{d^2\rho}{d\sigma^2} - \left(\frac{d\rho}{d\sigma} \right)^2 \right)}{\left(\frac{d\rho}{d\sigma} \right)^3}.
 \end{aligned}$$

Auch gelten die folgenden Sätze:

Damit die beiden Ebenenkurven, welche durch

$$\rho = \rho(t), \quad \bar{\rho} = \bar{\rho}(t)$$

gegeben sind, durch eine MÖBIUSSCHE Inversion und eine Bewegung ineinander überführbar seien, ist es notwendig und hinreichend, dass in den Punkten, in denen die Zuordnung durch

$$\frac{d\rho}{\rho} \frac{dt}{dt} = \frac{d\bar{\rho}}{\bar{\rho}^2} \frac{d\bar{t}}{d\bar{t}}$$

definiert ist, die Invariante I den gleichen Wert hat.

Damit sie durch eine Inversion und eine Bewegung ineinander überführbar seien, ist es notwendig und hinreichend, dass in den Punkten, in welchen die Zuordnung durch

$$\frac{d\rho}{\rho} \frac{d\sigma}{d\sigma} = \frac{d\bar{\rho}}{\bar{\rho}} \frac{d\bar{\sigma}}{d\bar{\sigma}}$$

definiert ist, die Invariante I den entgegengesetzt gleichen Wert hat.

Bezeichnet man

$$\int \sqrt{\pm \frac{d\rho}{d\sigma}} / \rho \, d\sigma$$

als Inversionsparameter p , so kann die Relation

$$\pm I = \varphi(p)$$

als die natürliche Gleichung der Ebenenkurve in der Inversionsgeometrie betrachtet werden.

Wenn die beiden Ebenenkurven mit den natürlichen Gleichungen

$$\rho = \rho(t), \quad \bar{\rho} = \bar{\rho}(\bar{t})$$

durch eigentliche LAGUERRESche Transformation ineinander überführbar sind, dann gilt

$$d\rho d\sigma = d\bar{\rho} d\bar{\sigma}.$$

Wenn sie durch uneigentliche Laguerresche Transformation ineinander überführbar sind, dann gilt⁽¹⁾

$$d\rho d\sigma = -d\bar{\rho} d\bar{\sigma}.$$

Das Gleiche gilt auch für die Invariante

$$I = \frac{5}{4} \frac{\left(\frac{d^2\rho}{d\sigma^2} - \left(\frac{d\rho}{d\sigma} \right)^2 \right)^2}{\left(\frac{d\rho}{d\sigma} \right)^3} + \frac{1}{4} \frac{d\rho}{d\sigma} \frac{1}{\rho^2} + \frac{1}{\frac{d\rho}{d\sigma}} -$$

$$\rho \frac{d^3\rho}{d\sigma^3} - 2 \left(\frac{d\rho}{d\sigma} \right) \frac{d^2\rho}{d\sigma^2} \rho - 2 \frac{d\rho}{d\sigma} \frac{d^2\rho}{d\sigma^2} + 2 \left(\frac{d\rho}{d\sigma} \right)^3$$

$$\left(\frac{d\rho}{d\sigma} \right)$$

$$- \frac{3}{2} \left(\frac{d^2\rho}{d\sigma^2} - \left(\frac{d\rho}{d\sigma} \right)^2 \right) \frac{d\rho}{d\sigma}.$$

wo die Differentiation in Bezug auf σ zu nehmen ist. Damit die beiden Ebenenkurven durch eine eigentliche LAGUERRESche Transformation ineinander überführbar seien, ist es notwendig und hinreichend, dass in den Punkten, in denen die Zuordnung durch⁽²⁾

- (1) KUBOTA, T.: Beiträge zur Inversionsgeometrie und LAGUERRESche Geometrie, Japanese Journ. of Math. Vol. I 1924 p 41
- (2) THOMSEN, G.: Über konforme Geo. II, Abh. aus dem Math. Sem. d. Hamb. Univ. IV Bd. S. 127.

$$d\rho d\sigma = d\bar{\rho} d\bar{\sigma}$$

definiert ist, auch die Beziehung⁽¹⁾

$$I = \bar{I}$$

gilt.

Damit die beiden Ebenenkurven durch eine uneigentliche LAGUERRESche Transformation ineinander überführbar seien, ist es notwendig und hinreichend, dass in den Punkten die Zuordnung durch⁽²⁾

$$d\rho d\sigma = -d\bar{\rho} d\bar{\sigma}$$

definiert ist, auch die Beziehung

$$I = -\bar{I}$$

gilt.

Man kann noch in meiner Arbeit⁽³⁾

$$\lambda \equiv \sigma$$

setzen.

(2)

Im folgenden vergleiche ich die Arbeit THOMSENS mit KUBOTAS Arbeit und setze dadurch den Fundamentalsatz in der Inversionsgeometrie in eine etwas modifizierte Form.

Unter der natürlichen Gleichung der ebenen Kurve \mathfrak{b} versteht man die fundamentale Beziehung zwischen dem Krümmungsradius und der Kurvenlänge

$$\rho = \rho(t),$$

- (1) MATSUMURA, S.: Beiträge zur Inversionsgeo. und LAGUERRE-Geo., Tôhoku Math. Journ. Vol. 37 (1933) p. 468.
- (2) THOMSEN, G.; Über konforme Geometrie II, Abhandlungen aus dem Math. Seminar der Hamb. Univ. Band IV (1925) S. 127.
- (3) KUBOTA, T.: Beiträge zur Inversionsgeometrie, The Science Reports of the Tôhoku Imp. Univ., Vol. XIII (1924-1925) p. 244.

wo ρ den Krümmungsradius und t die Kurvenlänge bedeutet, so folgt

$$\frac{dx}{dt} = \cos \sigma, \quad \frac{dy}{dt} = \sin \sigma,$$

$$\frac{d\sigma}{dt} = \frac{1}{\rho(t)}, \quad = \sigma \int \frac{dt}{\rho(t)},$$

wo wir die Zeichen in THOMSENS Arbeit benutzen,

Somit erhält man die Parameterdarstellung der Kurve:

$$x = \int \left\{ \cos \int \frac{dt}{\rho(t)} \right\} dt,$$

$$y = \int \left\{ \sin \int \frac{dt}{\rho(t)} \right\} dt.$$

Man bezeichnet die GAUSSSCHE Darstellung der komplexen Zahl durch den Punkt auf einer Ebene:

$$v = x + iy = \int \left\{ \cos \int \frac{dt}{\rho(t)} - i \sin \int \frac{dt}{\rho(t)} \right\} dt$$

$$= \int \exp. \left\{ i \int \frac{dt}{\rho(t)} \right\} dt.$$

Daraus erfolgt der

Satz: Die notwendige und hinreichende Bedingung dafür, dass die beiden Funktionen $v(t)$, $\bar{v}(\bar{t})$ durch eine Transformation der Form

$$\bar{v} = \frac{\alpha v + \beta}{\gamma v + \delta},$$

d. h.

$$-\int \bar{c} \bar{\xi}_o d\sigma = \frac{-\alpha \int c \xi_o d\sigma + \beta}{-\gamma \int c \xi_o d\sigma + \delta},$$

$$\xi_o = -\frac{d\bar{\xi}}{d\sigma}, \quad \alpha\delta - \beta\gamma \neq 0,$$

ineinander überführbar seien, wenn \bar{t} als eine gewisse Funktion von t betrachtet wird, ist die

$$\{v, t\} = \{\bar{v}, \bar{t}\},$$

wo

$$\{v, t\}, \quad \{\bar{v}, t\}$$

die Schwarzschen Ableitungen bedeuten und \bar{t} als eine gewisse Funktion von t betrachtet ist, wo

$$v_o = -c \xi_o,$$

$$\bar{v}_o = -\bar{c} \bar{\xi}_o,$$

$$\frac{d\sigma}{dt} = (\hat{\xi}, \xi_t) = 1/\rho.$$

(12) Über einen Parameter

Im folgenden wollen wir über KUBOTAS Parameter untersuchen mit den Zeichen in Thomsens⁽¹⁾ Arbeit.

Wir betrachten jetzt KUBOTAS Parameter⁽²⁾ dp .

Bei ihm ist

$$(1) \quad dp^2 = \frac{d\rho \cdot dt}{\rho} = d\rho \cdot d\sigma.$$

dp^2 ist eine Kurveninvariante.

Aus (1) folgt

$$(2) \quad \frac{d\rho}{dp} = \frac{dp}{d\sigma}$$

$$\text{d. h.} \quad d\rho : dp = dp : d\sigma,$$

also erfolgt der

Satz: dp ist das geometrische Mittel von $d\rho$ und $d\sigma$. Die Kurve, für welche

$$(3) \quad dp = 0$$

- (1) THOMSEN, G.: Über konforme Geometrie II, Abh. aus dem Math. Seminar der Hamb. Univ. IV Bd. (1925) S. 127.
- (2) KUBOTA, T.: On the Differential Invariants of the Laguerre Group, Proc. of the Cambr. Phil. Soc., Vol. 22 (1925).

gilt, wollen wir nach TAKASU⁽¹⁾ die KUBOTAS Minimalkurven nennen.

Aus (1) und (3) ergibt sich:

$$d\rho=0 \quad \text{oder} \quad d\sigma=0,$$

d. h. $\rho=\text{const.} \quad \text{oder} \quad \sigma=\text{const.}$

So folgt der

Satz: KUBOTAS Minimalkurven sind nicht anders als Kreis oder Gerade.

Wenn

$$d\bar{p}=dp$$

ist, so folgt

$$\sqrt{d\rho d\sigma}=\sqrt{d\bar{\rho} d\bar{\sigma}},$$

d. h. $d\rho d\sigma=d\bar{\rho} d\bar{\sigma}.$

Wenn $p=c\rho$, so kann man setzen: $p=c\sigma$,

Umgekehrt, wenn $p=c\sigma$ ist, so kann man setzen: $p=c\rho$, wo c eine Konstante ist.

Wenn $v(\sigma)$ Minimallinien oder isotrope Kurven sind, so geschieht

$$\left(-\frac{dv}{d\sigma}-\frac{dv}{d\sigma}\right)=0,$$

d. h. $\rho^2\left(-\frac{d\ddot{z}}{d\sigma}-\frac{d\ddot{z}}{d\sigma}\right)=0,$

oder

$$\left(\frac{d\ddot{z}}{d\sigma}-\frac{d\ddot{z}}{d\sigma}\right)=0, \quad (\rho^2 \neq 0).$$

d. h. eine Minimalkugelschar, wo sich die einander konsekutiven Kreise berühren.

N. B. (1) Wir haben

$$\tan\varphi=\frac{1}{3}\frac{d\rho}{ds}.$$

(1) TAKASU, T.: Differentialkugelgeo. XIII, The Science Reports of the Tōhoku Imp. Univ. Vol. XXII, (1933) p. 744.

Nach der SCHWARZschen Ungleichung haben wir :

$$\begin{aligned} L^2 &= (\int ds)^2 \leq (\int d\rho) \left\{ \int \left(\frac{ds}{d\rho} \right)^2 d\rho \right\} \\ &\leq P \left\{ \int \frac{1}{3 \tan \varphi} d\rho \right\} \\ &\leq P \cdot Q, \end{aligned}$$

wo $P = \int d\rho$, $Q = \int \frac{1}{3 \tan \varphi} d\rho$ ist.

(2) Wir haben

$$\tan \varphi = \frac{1}{3} \frac{d\rho}{ds};$$

die Formula⁽¹⁾ für P ist so mit

$$P = \rho \frac{d\rho}{ds} = 3\rho \tan \varphi$$

gegeben.

(13) Über Winkel, die die Kugeln mit einem Kreis enthalten

(1)

Wir betrachten uns nun zwei Paare von Richtungen :

$$(1) \quad \begin{cases} \cos^2 \varphi = T^{\alpha\beta} \rho_\alpha \rho_\beta = 0, \\ \cos^2 \bar{\varphi} = \bar{T}^{\alpha\beta} \rho_\alpha \rho_\beta = 0. \end{cases}$$

Die Bedingung dafür, dass die harmonische Trennung der beiden⁽¹⁾ Elementenpaare (I) ist, ist die

$$(2) \quad \theta(\cos^2 \varphi, \cos^2 \bar{\varphi}) = T^{11} \bar{T}^{22} - 2T^{12} \bar{T}^{12} + T^{22} \bar{T}^{11} = 0,$$

wo θ die Simultaninvariante von $\cos^2 \varphi, \cos^2 \bar{\varphi}$ ist.

Setzen wir $\cos^2 \varphi = 0$ und $\cos^2 \bar{\varphi} = 0$ in den Formen $T^2_\beta = 0$ und

(1) FOWLER, R. H.: The elementary differential Geometry of plane Curves, London, 1929, p. 42.

$$\bar{T}_p^2 = 0.$$

$$\text{Wenn } \cos^2 \varphi = -\lambda \cos^2 \bar{\varphi},$$

so

$$(3) \quad T_p^2 + \lambda \bar{T}_p^2 = 0,$$

wo λ ein Parameter ist.

Zwei Elementenpaare $T_p^2 = 0$, $\bar{T}_p^2 = 0$ bestimmen eine Involution, deren Paare in der Gleichung (3) enthalten sind.

Wir betrachten also eine Parameterverbindung von zwei quadratischen Formen wiederum als eine solche Form.

Setzt man die Diskriminante dieser Form gleich Null, so bestimmt die so entstehende Gleichung

$$(4) \quad (T^{11} + \lambda \bar{T}^{11})(T^{22} + \bar{T}^{22}) - (T^{12} + \lambda \bar{T}^{12})^2 = 0$$

die Parameter zwei in einen Punkt miteinander zusammenfallender Paare, der beiden Doppelemente.

Diese Funktion hat ihre Invarianteigenschaft für jeden beliebigen Wert des Parameters, ist also unabhängig von λ .

Daher sind schon die Koeffizienten der nach Potenzen von λ geordneten Diskriminante die Invarianten.

In der Tat lautet die Entwicklung

$$J_1 + \lambda \theta + \lambda^2 J_2$$

und führt also ausschliesslich auf die Diskriminanten der einzelnen Invarianten und die harmonische Simultaninvariante beider Paare.

Die Parameter der Doppelemente sind die absoluten Invarianten, nämlich

$$\lambda_1 = \frac{-\theta + \sqrt{\theta^2 - 4J_1J_2}}{2J_2}, \quad \lambda_2 = \frac{-\theta - \sqrt{\theta^2 - 4J_1J_2}}{2J_2}.$$

Deshalb haben die Doppelemente eine projektive Beziehung zu den gegebenen Elementenpaaren.

Das aus ihnen gebildete, der Involution nicht angehörige Elementenpaar ist also durch eine kovariante Gleichung darzustellen.

Das Quadrat derselben lässt sich unmittelbar als das Produkt

der die Doppelemente einzeln definierenden Gleichungen bilden, nämlich als

$$\begin{aligned} & 4_2(T_p^2 + \lambda_1 \bar{T}_p^2)(T_p^2 + \lambda_2 \bar{T}_p^2) \\ & \equiv 4_2(T_p^2)^2 - T_p^2 \bar{T}_p^2 + 4_1(T_p^2)^2 = 0. \end{aligned}$$

Es ist leicht zu bestätigen, dass diese biquadratische Kovariante $-F^2$ gleich ist, wo

$$F \equiv \begin{vmatrix} T^{11}\rho_1 + T^{12}\rho_2 & T^{12}\rho_1 + T^{22}\rho_2 \\ \bar{T}^{11}\rho_1 + \bar{T}^{12}\rho_2 & \bar{T}^{12}\rho_1 + \bar{T}^{22}\rho_2 \end{vmatrix}$$

ist.

Also bilden die Doppelemente das gemeinsame, mit den Involutionspaaren harmonische Paar.

In einer Involution können die im allgemeinen getrennten Doppelemente zu den Fundamentelementen gewählt werden.

Bezeichnen wir sie als z_1, z_2 mit

$$T_p^2 + \lambda_1 \bar{T}_p^2 = z_1^2, \quad T_p^2 + \lambda_2 \bar{T}_p^2 = z_2^2, \quad (\lambda_1 \neq \lambda_2),$$

so kann die Gleichung der Involution in der Gestalt

$$z_1^2 - \frac{\lambda - \lambda_1}{\lambda - \lambda_2} z_2^2 = 0$$

geschrieben werden, aus der die harmonische Trennung der Paare durch die Doppelemente sofort hervorgeht.

Die Doppelemente fallen miteinander zusammen ($\lambda_1 = \lambda_2$) und die Involution ist parabolisch, wenn die Invariante

$$\theta^2 - 4A_1A_2$$

verschwindet.

(2)

Betrachten wir

$$(1) \quad \cos^2 \varphi = T^{*1} \rho_a \rho_b$$

wieder,⁽¹⁾ und setzen

$$(2) \quad \phi = T^{\sigma\tau} \rho_\sigma \rho_\tau, \quad \psi = k^{\sigma\tau} \rho_\sigma \rho_\tau,$$

wo

$$(3) \quad T = |T^{\sigma\tau}| = \begin{vmatrix} T^{11} & T^{12} \\ T^{12} & T^{22} \end{vmatrix} \neq 0,$$

$$T^{ij} = T^{ji}, \quad h^{ij} = h^{ji},$$

$$T_{\sigma\tau} h^{\sigma\tau} = T_{22} h^{11} + 2T_{12} h^{12} + T_{11} h^{22} = 0,$$

so nennen wir ψ die Apolarität zu ϕ .

In unserem Falle werden zwei Kugeln, die mit

$$(4) \quad \phi = 0$$

bestimmt werden, harmonisch getrennt durch die Kugeln, die mit

$$(5) \quad \psi = 0$$

bestimmt werden.

Nun betrachten wir die kubische Grundform

$$(6) \quad x = k^{\sigma\tau\rho} \rho_\sigma \rho_\tau \rho_\rho,$$

wo

$$(7) \quad k^{ijl} = k^{jil} = k^{lji}; \quad i, j, l = 1, 2,$$

$$(8) \quad T^{\sigma\tau} k_{\sigma\tau i} = 0 \quad (i = 1, 2);$$

Wir nennen x die Apolarität zu ϕ .

(8) ist invariant durch die lineare Transformation von ρ .

Nun betrachten wir eine Transformation, wodurch

$$(9) \quad k_{112} = k_{122} = 0$$

ist. x wird hier

$$(10) \quad k^{111}(\rho_1)^3 + k^{222}(\rho_2)^3.$$

Die Hessesche Kovariante von (10) ist

$$(11) \quad k^{111} k^{222} \rho_1 \rho_2,$$

(1) MATSUMURA, S.: Beiträge zur Geo. der Kreise und Kugeln (VII), Mem. of the Fac. of Sci. and Agr., Taihoku Imp. Univ. Vol. V, p. 379.

wo

$$k^{111} \neq 0, \quad k^{222} \neq 0.$$

Die Gleichung, die (11) zu Null setzt, stimmt mit $\varphi=0$ überein.

(3)

Nehmen wir

$$\cos^2 \varphi = T^{\alpha\beta} \rho_\alpha \rho_\beta.$$

In der binären quadratischen Form

$$\varphi(\rho_1, \rho_2) = T^{11} \rho_1^2 + 2T^{12} \rho_1 \rho_2 + T^{22} \rho_2^2 = (T^{11}, T^{12}, T^{22})$$

der positiven Discriminante

$$D = 4((T^{12})^2 - T^{11}T^{22}) = R^2$$

sind

$$r = -\frac{R + 2T^{12}}{2T^{22}} = \frac{2T^{11}}{R - 2T^{12}},$$

$$s = \frac{R - 2T^{12}}{2T^{22}} = -\frac{2T^{11}}{R + 2T^{12}}$$

die Wurzeln.

Man nennt r die erste, s die zweite Wurzel.

Die Form $\varphi = \cos^2 \varphi$ nimmt also den Wert 0 nicht an, T^{11} und T^{22} sind stets von 0 verschieden, r und s weder 0 noch ∞ .

Zwei Formen $\varphi(\rho_1, \rho_2)$ und $\varphi'(\rho'_1, \rho'_2)$ sind äquivalent, wenn φ in φ' durch eine Substitution

$$(1) \quad \begin{cases} \rho_1 = \alpha \rho'_1 + \beta \rho'_2, \\ \rho_2 = \gamma \rho'_1 + \delta \rho'_2 \end{cases} \quad P = \begin{pmatrix} \alpha & \beta \\ \gamma & \delta \end{pmatrix},$$

übergeht, deren Koeffizienten ganze Zahlen sind und deren Determinante

$$\alpha\delta - \beta\gamma = +1$$

ist.

Ist r' die erste Wurzel ϕ' , so ist

$$(2) \quad r = \frac{\gamma + \delta r'}{\alpha + \beta r'}.$$

Mit (δ) bezeichnen wir die spezielle Substitution

$$(3) \quad (\delta) = \begin{pmatrix} 0 & -1 \\ +1 & \delta \end{pmatrix}, \quad r = -\delta - \frac{1}{r'}.$$

Durch diese geht

$$\cos^2 \varphi \equiv \phi = (T^{11}, 2T^{12}, T^{22})$$

in die benachbarte Form

$$\cos^2 \varphi_1 \equiv \phi_1 = (T_1^{11}, 2T_1^{12}, T_1^{22})$$

über, wo

$$(4) \quad \begin{aligned} T_1^{12} + T^{12} &= T_1^{11} \delta, \\ T_1^{22} &= T^{11} - 2T^{12} \delta + T_1^{11} \delta^2 \\ &= T^{11} + \delta(T^{12} - T_1^{12}) \end{aligned}$$

ist.

(14) Über Laguerre-Geometrie

Ein orientierter Kreis im R_2 mit dem Zentrum (A, B) und Radius R hat offenbar die folgende Tangentialgleichung:

$$(1) \quad Au + Bv - q \sin \alpha - R = 0, \quad (u^2 + v^2 = 1), \quad p = q \sin \alpha, \\ (p \text{ Tangentialpolarkoordinaten!}),$$

wo α den Winkel zwischen Tangente und Radius bezeichnen.

Wir schreiben (1) wie folgt um:

$$(2) \quad Au + Bv + (-q \sin \alpha) + iR(i\sqrt{u^2 + v^2}) = 0.$$

Dann heissen

$$(3) \quad \xi_1 = A, \quad \xi_2 = B, \quad \xi_3 = iR, \quad \xi_4 = 1,$$

die LAGUERRESche Kreiskoordinaten des orientierten Kreises (1) und

$$(4) \quad u^1 = u, \quad u^2 = v, \quad u^3 = i\sqrt{u^2 + v^2}, \quad u^4 = -q \sin \alpha$$

die LAUGERRESCHEN Geradenkoordinaten der orientierten Geraden $(u, v, q \sin \alpha)$.

Durch diese Bezeichnung wird aus (1):

$$(5) \quad (u\xi) = 0.$$

Wir betrachten ein ein-parametriges System von Kreisscharen⁽¹⁾

$$(6) \quad \xi = \xi(s).$$

Zwei benachbarte L-Kreise des Systems (6) haben den L-Abstand ds , nämlich

$$(7) \quad ds^2 = (d\xi \, d\xi).$$

Drei konsekutive L-Kreise bestimmen das Feld

$$(8) \quad \chi = \rho || \xi \ddot{\xi} \ddot{\xi} ||,$$

wo

$$\dot{\xi} = \frac{d\xi}{d\sigma} \quad \text{u. s. w..}$$

Ist dS das durch das Feld χ beschriebene Winkelement, so wird

$$(9) \quad dS = (d\chi \, d\chi).$$

Für die gemeinsamen Geraden n, \bar{n} haben wir

$$(l\chi = m\chi', \quad l\chi + m\chi') = 0,$$

wo

$$\chi' = \frac{d\chi}{dS}.$$

Wir können die folgenden Systeme von Frenetschen und Daulfrenetschen Formeln erhalten.

- (1) TAKASU, T.: LAGUERRE-geometrische Verallgemeinerung der Kurventheorie in der Ebene, The Science Reports of the Tôhoku Imp. Univ. Series I, Vol. XXII, No. 4.

	u	\bar{u}	t	χ		u	\bar{u}	t	χ
u	0	1	0	$\frac{i}{v}$	u	0	1	0	$\frac{i}{v}$
\bar{u}	1	0	0	$-\frac{i}{v}$	\bar{u}	2	0	0	$-\frac{i}{v}$
$\frac{du}{d\theta}$	0	0	1	0	$\frac{d\bar{u}}{ds}$	0	0	$-\frac{1}{vR}$	0
$\frac{dt}{d\theta}$	-1	$-\mu^2$	0	0	$\frac{dt}{ds}$	$\frac{1}{vR}$	$\frac{v^2}{R}$	0	0
$\frac{d\bar{t}}{d\theta}$	0	0	$-\frac{1}{P}$	0	$\frac{d\bar{t}}{ds}$	0	0	-1	0
$\frac{d\chi}{d\theta}$	$i \frac{d}{d\theta} \left(\frac{1}{v} \right)$	$-\frac{dv}{d\theta}$	0	0	$\frac{d\chi}{ds}$	$i \frac{d}{ds} \left(\frac{1}{v} \right)$	$-\frac{dv}{ds}$	0	0

wo

$$d\theta = (du \, du)^{\frac{1}{2}}, \quad t = \frac{du}{d\theta}, \quad P = \frac{d\theta}{ds} = \frac{1}{vR}, \quad -v \frac{d\theta}{ds} = \frac{1}{R}.$$

Natürlich kann man wieder die Formeln in Takasus Arbeit⁽¹⁾ herleiten. Auch kann man sie in Kugelgeometrie erweitern.

N. B. Anstatt (1) kann man

$$Au + Bv - q \sin \alpha - R - k = 0$$

setzen, wo k eine Konstante ist.

(15) Über Tragheitskreise und Kugeln

(1)

Es seien

$$x = x(s), \quad y = y(s)$$

(1) TAKASU, T.: op. cit.

eine gegebene konvexe geschlossene ebene Kurve, dann nennen wir den so invariant verbundenen Punkt

$$(X, Y),$$

dass das darauf bezogene Trägheitsmoment

$$(1) \quad I(P) = \oint \left\{ (x-X)^2 + (y-Y)^2 \right\} \frac{ds}{\rho},$$

ein Minimum werde, den Krümmungsschwerpunkt der konvexen geschlossenen Kurve, wo ρ^{-1} die Krümmung und ds das Bogenelement ist.

Aus

$$(2) \quad \frac{\partial I}{\partial X} = 0, \quad \frac{\partial I}{\partial Y} = 0,$$

erfolgt⁽¹⁾

$$\oint \frac{(x-X)ds}{\rho} = 0, \quad \oint \frac{(y-Y)ds}{\rho} = 0,$$

$$\text{d. h. } (3) \quad X = \oint \frac{x ds}{\rho} / \int \frac{ds}{\rho}, \quad Y = \oint \frac{y ds}{\rho} / \int \frac{ds}{\rho}.$$

Es seien

$$x = x(s), \quad y = y(s)$$

eine gegebene konvexe geschlossene ebene Kurve, dann nennen wir den so invariant verbundenen Kreis

$$(4) \quad (\xi - X)^2 + (\eta - Y)^2 - r^2 = 0,$$

dass das darauf bezogene Trägheitsmoment

$$(5) \quad I(k) = \oint \left\{ (x-X)^2 + (y-Y)^2 - r^2 \right\} \frac{ds}{\rho}$$

(1) NAKAJIMA, S.: The Circle and the straight Line nearest to n given Points, n given straight Lines or a given Curve, Tôhoku Math. Journ. Vol. 19 (1921) p. 11.

SU, B.: On the Curvature-Axis of the convex closed Curve, The Science Reports of the Tôhoku Imp. Univ. Vol. XVII (1928) p. 35.

TAKASU, T.: Über einige Gegenstücke des STEINERschen Krümmungsschwerpunktes, I, Tôhoku Math. Journ. Vol. 32 (1930), p. 111.

ein Minimum werde, den Krümmungsschwerkreis der konvexen geschlossenen Kurve, wo ρ^{-1} die Krümmung, ds das Bogenelement, r konstant und das Zentrum (X, Y) veränderlich ist.

Sucht man denjenigen Kreis (3), wo das Trägheitsmoment extrem wird, so ergeben sich nach

$$(6) \quad -\frac{\partial I}{\partial X} = 0 \quad \text{und} \quad \frac{\partial I}{\partial Y} = 0,$$

die folgenden Bedingungen:

$$(7) \quad \begin{cases} \int (x-X) \frac{ds}{\rho} = 0, \\ \int (y-Y) \frac{ds}{\rho} = 0. \end{cases}$$

Aus (7) folgt

$$(8) \quad \begin{cases} X = \int \frac{x ds}{\rho} / \int \frac{ds}{\rho}, \\ Y = \int \frac{y ds}{\rho} / \int \frac{ds}{\rho}. \end{cases}$$

(8) ist unser Mittelpunkt des gesuchten Kreises, so ist

$$\left(\xi - \int \frac{x ds}{\rho} / \int \frac{ds}{\rho} \right)^2 + \left(\eta - \int \frac{y ds}{\rho} / \int \frac{ds}{\rho} \right)^2 = r^2$$

der Kreis.

(7) ist der sogenannte STEINERSche Krümmungsschwerpunkt.⁽¹⁾

(2)

Es sei eine gegebene konvexe geschlossene Fläche im R_3 , dann nennen wir diejenige Kugel

$$(\xi - X)^2 + (\eta - Y)^2 + (\zeta - Z)^2 - r^2 = 0,$$

wo das darauf bezogene Trägheitsmoment

⁽¹⁾ Werke II, pp. 97-159, oder CRELLEsche Journal 21 S. 33-63 und pp. 101-133.

$$(1) \quad J(k) = \iiint \left\{ (x-X)^2 + (Y-y)^2 + (Z-z)^2 - r^2 \right\} \frac{d\omega}{\rho_1 \rho_2}$$

ein Minimum wird, die Krümmungsschwerkugel der konvexgeschlossenen Kurve, wo $(\rho_1 \rho_2)^{-1}$ die Krümmung, $d\omega$ das Flächenelement, konstant und das Zentrum (X, Y, Z) veränderlich ist.

Aus

$$-\frac{\partial J}{\partial X} = 0, \quad -\frac{\partial J}{\partial Y} = 0, \quad -\frac{\partial J}{\partial Z} = 0,$$

folgt

$$(2) \quad \begin{aligned} & \left(\iint (x-X) \frac{d\omega}{\rho_1 \rho_2} = 0, \right. \\ & \iint (y-Y) \frac{d\omega}{\rho_1 \rho_2} = 0, \\ & \left. \iint (z-Z) \frac{d\omega}{\rho_1 \rho_2} = 0. \right) \end{aligned}$$

so wird der Mittelpunkt unserer Kugel mit (2) gegeben.

IONIC BALANCE IN AIR AND NUCLEI OVER OCEAN

(With 4 Text Figures)

Katsuyoshi SHIRATORI

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(I)

In this paper we have studied the ionic balance in air and found the zonal distribution of nuclei over the ocean under the condition of equilibrium between small ions and large ions from the results of observations of the "CARNEGIE".⁽¹⁾

(II) Rate of Loss of Ions.

The rate of destroying ions is proportional to the product of the concentrations of the two kinds of small ions, positive and negative; therefore the rate of loss of ions can be written

$$\frac{dn}{dt} = q - \alpha n^2 \quad (1)$$

This is the ordinary square law in air free from nuclei-content. The q is the rate of productions of ions per unit volume, n the number of either positive or negative small ions and α the recombination coefficient of small ions.

But as the ordinary atmosphere contains plenty of nuclei. the equation becomes

$$\begin{aligned} \frac{dn}{dt} &= q - \alpha n^2 - \gamma n N \\ &= q - \alpha n^2 - \beta n \\ &= q - \beta' n. \end{aligned} \quad (2)$$

$$\beta = \gamma N, \quad \beta' = (a n + \beta)$$

where N is the number of large ions or nuclei, γ is the recombination co-efficient between small ions and large ions. If air contains plenty enough of nuclei β is practically equal to β' and β' is the rate of loss of light ions with all kinds of ions and nuclei. This relationship is called "SCHWEIDLER'S LINEAR⁽⁴⁾ Combination Law" which generally holds good in practical cases. Of course β' is not constant, and depends on the number of nuclei. Analogous to radioactive disintegration the average life of small ions is defined by

$$\frac{1}{\beta'} = \theta.$$

Introducing the recombination co-efficients between small ions (n_1, n_2) and large ions (N_1, N_2) and uncharged nuclei (N_0) J. J. NOLAN⁽³⁾ and his co-workers have shown the equation

$$-\frac{dn_1}{dt} = q - a n_1 n_2 - (\gamma_{10} N_0 + \gamma_{12} N_2) n_1 \quad (3)$$

$$-\frac{dn_2}{dt} = q - a n_1 n_2 - (\gamma_{20} N_0 + \gamma_{21} N_1) n_2 \quad (4)$$

The suffix "1" and "2" indicates "positive" and "negative" respectively, and γ_{10} or γ_{20} is the recombination co-efficients of small ions with uncharged nuclei and γ_{12} or γ_{21} that of small ions with oppositely charged nuclei. As shown by NOLAN⁽³⁾ or MC-CLELLAND⁽⁵⁾ and KENNEDY⁽⁶⁾ N_1 and N_2 are practically equal.

Now putting

$$\left. \begin{aligned} \frac{\gamma_{12}}{\gamma_{10}} &= \frac{\gamma_{21}}{\gamma_{20}} = l \\ -\frac{\gamma_{20}}{\gamma_{10}} &= -\frac{\gamma_{21}}{\gamma_{12}} = p \end{aligned} \right\} \text{and} \left. \begin{aligned} l &= -\frac{N_0}{N_{12}} \\ p &= -\frac{n_1}{n_2} \end{aligned} \right\} \quad (5)$$

and neglecting the term $a n_1 n_2$ for the free atmosphere, we have the equilibrium equation

$$\begin{aligned} q &= 2 \gamma_{12} n_1 N_2 \\ &= 2 \gamma_{21} n_2 N_1 \end{aligned} \quad (6)$$

In 1929 P. J. NOLAN⁽⁷⁾ and C. O'BROLCHAIN found that γ_{12} is not constant. Though the direct proportionality between q and n holds good, the value of γ_{12} decreased for air of high nucleus content and increased for air of low nucleus content, and P. J. NOLAN⁽⁸⁾ reached the conclusion that the variation of γ_{12} occurs in such a way that $\gamma_{12}\sqrt{N}$ is approximately constant. Therefore, by introducing a new co-efficient τ for which he gave the character of constant, the new equilibrium equation has been proposed by him, as:—

$$q = \alpha n^2 + \tau n \sqrt{N} \quad (7)$$

It may be noted that this new equation implies that collisions between small ions and nuclei may not be held by the law of mass action, and thus the collision-frequency between them may not be proportional to the product of their concentration. When the nuclei are sufficient plenty the equation becomes $q = \tau n \sqrt{N}$, and NOLAN⁽⁸⁾⁽⁹⁾ gave the value $\tau = 55 \times 10^{-5}$.

If these above equations of ionic equilibrium of atmosphere are all equivalent, then they must be brought into relationship to each other by the equation

$$\beta' = 2 \gamma_{12} N_2 = \tau \sqrt{N} \quad (8)$$

(III) Relation between Recombination Co-efficients of Small Ions with Nuclei

Here we have the equation for the equilibrium state of small ions in the free atmosphere,

$$\left. \begin{aligned} \frac{dn_1}{dt} &= q - \alpha n_1 n_2 - \gamma_{10} n_1 N_0 - \gamma_{12} n_1 N_2 = 0 \\ \frac{dn_2}{dt} &= q - \alpha n_1 n_2 - \gamma_{20} n_2 N_0 - \gamma_{21} n_2 N_1 = 0 \end{aligned} \right\} \quad (9)$$

likewise the equations for the equilibrium state of large ions or charged nuclei and uncharged nuclei may be

$$\left. \begin{aligned} \frac{dN_1}{dt} &= \gamma_{10} n_1 N_0 - \gamma_{21} n_2 N_1 = 0 \\ \frac{dN_2}{dt} &= \gamma_{20} n_2 N_0 - \gamma_{12} n_1 N_2 = 0 \end{aligned} \right\} \quad (10)$$

of course it is assumed here that the recombination co-efficient between large ions is negligible a small one.

Put

$$\frac{n_1}{n_2} = p \quad \frac{N_0}{N_1} = l_1 \quad \frac{N_0}{N_2} = l_2 \quad (11)$$

and

$$N = N_0 + N_1 + N_2 .$$

As shown by J. J. NOLAN⁽³⁾⁽⁴⁾⁽⁶⁾ and his⁽⁸⁾⁽¹⁰⁾⁽¹¹⁾ workers or McCLELLAND and KENNEDY⁽⁸⁾⁽⁶⁾ or O'BROLCHAIN⁽¹⁰⁾ the fact that in general N_1 is almost equal to N_2 may be practically true. Consequently

$$N_1 = N_2 \quad \text{and} \quad l_1 = l_2 = l$$

then

$$\left. \begin{aligned} N &= N_0 \left(\frac{l+2}{l} \right) = N_{12} (l+2) \\ N_0 &= N \left(\frac{l}{l+2} \right) = N_{12} l \\ N_{12} &= N \left(\frac{1}{l+2} \right) = N_0 \left(\frac{1}{l} \right) \end{aligned} \right\} \quad (12)$$

For a steady state of ionic atmosphere the equations (9) and (10) become

$$\begin{aligned} \left(q - \frac{a}{p} n_1^2 \right) &= (\gamma_{10} N_0 + \gamma_{12} N_2) n_1 \\ &= (\gamma_{20} N_0 + \gamma_{21} N_1) \frac{n_1}{p} \\ \gamma_{10} &= \gamma_{21} \frac{1}{pl} \\ \gamma_{20} &= \gamma_{12} \frac{p}{l} \end{aligned} \quad (13)$$

$$p \gamma_{10} (N_0 - l N_{12}) = \gamma_{20} (N_0 - l N_{12})$$

therefore

$$p \gamma_{10} = \gamma_{20} \quad (14)$$

From these relations we are able to get the following connections between the recombination co-efficients of small ions with nuclei.

$$\left. \begin{aligned} \gamma_{10} &= \frac{\gamma_{20}}{p} = \frac{\gamma_{12}}{l} = \frac{\gamma_{21}}{lp} \\ \gamma_{20} &= p \gamma_{10} = \gamma_{12} \frac{p}{l} = \frac{\gamma_{21}}{l} \\ \gamma_{12} &= \gamma_{10} l = \gamma_{20} \frac{l}{p} = \frac{\gamma_{21}}{p} \\ \gamma_{21} &= \gamma_{10} p l = \gamma_{20} l = \gamma_{12} p \end{aligned} \right\} \quad (15)$$

In preference to LANGEVIN'S⁽¹¹⁾ equation concerning atmospheric air with nucleus content, WHIPPLE⁽¹²⁾ has suggested a new relation as follows :

$$\left. \begin{aligned} \gamma_{12} - \gamma_{10} &= 4 \pi e w_1 \\ \gamma_{21} - \gamma_{20} &= 4 \pi e w_2 \end{aligned} \right\} \quad (16)$$

The "w" is the mobility of small ions.

Substituting this relation into the equation (15) for the equilibrium condition of ionic atmosphere, then

$$\gamma_{12} = 4 \pi e w_1 \frac{l}{(l-1)} \quad (17)$$

moreover, we can also get

$$\frac{w_1}{w_2} = \frac{1}{p} \quad \therefore \quad p = \frac{w_2}{w_1} = \frac{\gamma_{20}}{\gamma_{10}} = \frac{\gamma_{21}}{\gamma_{12}} \quad (18)$$

Thus this condition results that in a equilibrium ionic atmosphere the ratio of the numbers of small ions of both signs is inversely proportional to the ratio of the mobilities of both ions. Therefore if Whipple's new equation can be applicable, then in equilibrium state it thus becomes that the product of the number of ions with their mobility is equal for both positive and negative small ions.

$$\left. \begin{array}{l} n_1 w_1 = n_2 w_2 \\ \text{and } w_1 \gamma_{20} = w_2 \gamma_{10} \end{array} \right\} \quad (19)$$

(IV) Ionic Equilibrium.

From equation (13) the equilibrium ionic state of air is

$$\left(q - \frac{a}{p} n_1^2 \right) = 2 \gamma_{12} n_1 N_{12} \quad (20)$$

so that

$$\gamma_{12} = \frac{\left(q - \frac{a}{p} n_1^2 \right) (l+2)}{2 n_1 N}$$

The value of γ_{12} should depend on the number of nuclei in air. If as NOLAN's new equation is equivalent with the ordinary one like as the equation (8), then

$$2\gamma_{12} \frac{N}{l+2} = \tau \sqrt{N}$$

or

$$\gamma_{12} \sqrt{N} = \tau \left(1 + \frac{l}{2} \right) \quad (21)$$

But according to NOLAN's⁽⁶⁾ conclusion the value of $\gamma_{12} \sqrt{N}$ may be deemed as a constant, thus by this fact we naturally come to be satisfied with the condition, as an

$$l = \text{constant}$$

Concerning the value of "l" J. J. NOLAN⁽¹³⁾ and P. J. NOLAN⁽¹⁴⁾ obtained a value 2.2 at Glencree in a country district near Dublin; GOCKEL⁽¹⁵⁾ found a value 2.4; HESS⁽¹⁶⁾ showed in Heligoland a value 2.2; O'BROLCHAIN found a value 2.67 on the outskirt of Graz; and J. SCHOLZ obtained in Westerland a value 3.4 for land wind and 1.1 for sea wind.

Using the individual data obtained by NOLANS,⁽¹³⁾ O'BROLCHAIN⁽¹⁶⁾ and SCHOLZ,⁽¹⁷⁾⁽¹⁸⁾ the graphs of Fig. 1A and 1B are made by plotting

N_0 against N . Fig. 1A as a graph for N is less than 10,000 while Fig. 1B for N more than 10,000. As clearly seen in the figure, almost all points are nearly represented by a straight line, which slope is $\frac{N_0}{N} = 0.56$. So that from this straight line it may be said that the " l " is nearly constant and 2.6 as the most reliable value in fair agreement with O'BROLCHAIN's one.

$$\frac{N_0}{N_{2.1}} = 2.6$$

If we take 2.6 for the value of l and 55×10^{-8} for ζ then

$$\gamma_{12} \sqrt{N} = 12.65 \times 10^{-4}$$

and assuming $N=10,000$ we have

$$\gamma_{12} = 12.65 \times 10^{-6}$$

or $N=1,3000$ then

$$\gamma_{12} = 9.7 \times 10^{-6}$$

These values of γ_{12} are sufficiently acceptable and well agree with the value obtained directly from the experiment.

Comparing NOLAN's constant τ with WHIPPLE's new formula, it can be written as:

$$\frac{\tau}{8\pi e} = w_1 \frac{l\sqrt{N}}{(l+2)(l-1)}$$

$$w_1 N = \frac{\tau \sqrt{N} (l+2)(l-1)}{8\pi l e}$$

As the right hand side may be deemed to be constant, consequently the mobility of small ions may be said to be not constant and varies according to the number of the nuclei under the condition that the product of the mobility and the nucleus number is constant.

Let it be noted that the value of the mobility depends on pressure and humidity and its peculiar variation was found in air of different humidity by NOLAN and NEVIN.⁽¹⁰⁾

V Nuclei in Ocean.

From the ordinary square law of ionic equilibrium HESS⁽²⁰⁾ found a value 970 as the number of small ions over ocean. But this value seems to be somewhat higher than the value actually obtained by the CARNEGIE-expedition. The reason of this discrepancy must be due to the effect of distribution of nuclei over the sea. Therefore if we calculate the number of nuclei over ocean by the equation

$$N = \left(q - \frac{a}{p} n_1^2 \right) \left(\frac{l+2}{2 \gamma_{12} n_1} \right)$$

where a is 1.6×10^{-6} , and if we adopt the value of γ_{12} from the equation (21), then the relation is just equivalent with NOLAN's new formula and as the value l is deemed to be 2.6, the number of nuclei is obtained

$$N = \left(\frac{q - \frac{1.6}{p} n_1^2 \cdot 10^{-6}}{55 n_1 \cdot 10^{-5}} \right)^2$$

and the number of large ions or charged nuclei is

$$N_{12} = \frac{N}{4.6}$$

Table I shows the result obtained by this equation and the map is the zonal distribution of nuclei over the sea.

The original material are averaged for each square of 5 degrees in latitude and 10 degrees in longitude and such mean values are in each column of the table.

Of course these values show only the general distribution and the map is the zonal distribution of nuclei over the sea.

The original material are averaged for each square of 5 degree in latitude and 10 degrees in longitude and such mean values are in each column of the table.

Of course the values show only the general distribution and which might vary to some considerable degree by season, and by

the meteorological conditions, because the materials for this calculation were not obtained in the same season, as shown in the first column of the table and also are not corrected into the same meteorological conditions: but still they suffice to give the outline of the distribution of the nuclei and ions over the ocean.

As clearly seen in the map, the nuclei rapidly increases by approaching nearer to land. All sea near shore land gives the contents far more than one thousand of nuclei in a unit volume of air, though the central part of ocean contains only more or less one hundred of nuclei. It also seems that the air over the busy routes of steamers contains comparatively much more nuclei even in the central part of oceans. The North Atrantic Ocean is more nucleus than the North Pacific.

The main least nucleus parts are the western central part of the North Pacific, the central part of the Indian ocean and the western part of the South Atlantic and the far eastern part from New Zealand in the South Pacific.

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Table I. Zonal Distribution of Nuclei

 f = Numbers of the observations used for taking the mean. n = Numbers of small ions per unit volume. R = Ions/cc.,/sec. produced by penetrating radiation. η^0 = Ions/cc.,/sec. produced by radioactive content. q = $(R + \eta^0)$ ions/cc.,/sec. produced by ioniser. N = Numbers of total nuclei charged and unchanged. $N_{1,2}$ = Number of charged nuclei.

Longitude	f	Date	$n +$	$n -$	$\frac{n +}{n -}$	R	η^0	q	Press.	Temp.	Humid.	W.V.	N	$N_{1,2}$
No. 1														
(N. 0.0°-4.9°)														
60.0-	1	1920 June	624	457	1.36	4.08	0.44	4.52	760	27.5	72	3	262	57
80.0-	4	1920 July	630	520	1.21	3.96	1.63	5.59	758	28.0	82	5	324	70
160.0-	6	1915 Sept. Oct. }	626	502	1.25	3.36	0.18	3.54	758	30.2	72	2	116	25
180.0-	3	1916 June	388	279	1.32	2.99	0.05	3.04	753	23.1	79	4	306	67
260.0-	5	1915 Apr.	627	444	1.41	3.17	1.32	4.49	759	29.0	79	2	207	45
270.0-	6	1915 Apr.	526	474	1.11	3.06	1.62	4.68	757	28.5	76	3	338	74
280.0-	1	1918 Apr.	441	365	1.21	3.81	2.12	5.93	759	27.0	79	3	829	180
350.0-	7	1919 Dec	273	216	1.26	4.56	2.12	6.63	758	23.0	79	2	2959	643
(N. 5.0°-9.9°)														
50.0-	1	1920 June	612	529	1.16	4.32	0.62	4.94	758	28.5	78	6	246	54
60.0-	2	1920 June	605	534	1.14	4.20	0.58	4.78	757	28.3	77	4	246	54

70.0-	1	1920	June	637	554	1.15	4.76	2.46	7.22	758	27.5	82	4	547	116
160 0-	3	1915	Sept.	662	561	1.18	3.39	0.20	3.59	757	31.3	72	3	108	24
180.0-	1	1916	June	394	367	1.07	2.75	0.00	2.75	757	28.7	77	5	207	45
200 0-	2	1921	Jan.	624	497	1.26	3.25	0.48	3.73	757	28.4	78	4	136	30
250 0-	2	1915 1916	May Nov. }	781	686	1.14	3.68	3.11	6.79	758	28.9	75	3	292	64
160-	5	1915	Apr.	718	571	1.26	3.31	1.38	4.69	759	29.0	80	2	158	34
270-	1	1915	Apr.	491	359	1.37	3.04	3.35	6.39	758	30.5	71	0	778	169
280-	1	1918	Apr.	627	621	1.01	3.73	3.69	7.42	760	26.3	86	3	590	128
340-	8	1919	Nov. } Dec }	340	331	1.03	4.41	7.32	11.73	758	28.8	72	1	5852	1272
No. 2															
(N 10°-15°)															
60-	2	1920	June	410	389	1.06	4.16	0.55	4.71	755	28.9	77	6	590	128
140-	2	1916	July	698	605	1.15	2.70	0.54	3.24	770	28.2	84	3	81	18
160-	5	1915	Sept.	671	543	1.24	3.46	0.26	3.72	758	30.3	75	2	98	21
170-	1	1916	July	569	568	1.00	3.87	0.00	3.87	759	27.2	85	2	169	37
180-	1	1916	June	703	612	1.15	3.21	0.00	3.21	758	28.0	82	5	59	13
230-	2	1915	May	737	625	1.18	5.98	1.58	7.56	764	25.9	70	4	428	93
240-	3	1915 1916	May Nov. }	498	460	1.19	3.15	2.53	5.68	760	27.0	79	4	571	124
250-	4	1915	May	638	602	1.06	3.30	1.57	4.87	758	29.4	81	4	222	48
270-	1	1918	May	376	302	1.24	4.17	1.37	5.54	760	29.5	70	4	1049	228
280-	2	1918	May	380	326	1.16	6.17	1.54	7.71	761	28.8	75	5	1980	431

170-	1	1915 Sept.	610	580	1.15	3.51	1.64	5.15	763	28.2	81	1	292	64
180-	3	1915 July	531	477	1.12	3.62	0.43	4.05	767	24.9	70	4	234	51
220-	1	1921 Apr.	846	781	1.08	3.32	2.17	5.49	763	16.2	72	3	136	30
230-	2	1918 May	325	275	1.10	3.66	1.42	5.08	767	27.3	72	4	1169	254
340-	3	1919 Nov.	803	616	1.30	3.60	1.89	5.49	760	22.1	80	3	171	37
(N. 30°-35°)														
160-	1	1915 Aug.	497	383	1.20	3.41	0.86	4.27	760	23.6	79	4	81	18
170-	3	1915 Aug. } Sept. }	672	504	1.33	3.43	1.91	5.34	761	25.2	89	3	262	57
190-	1	1915 July	625	518	1.20	3.66	0.15	3.81	765	23.0	87	4	136	30
200-	2	1921 May	809	609	1.33	3.15	2.42	5.57	758	18.7	76	3	182	40
210-	4	1921 May	668	544	1.23	3.15	3.01	6.16	759	17.4	74	4	357	78
220-	2	1921 Apr. } May }	601	480	1.25	3.02	1.10	4.12	764	17.8	74	4	182	40
230-	1	1921 Mar.	653	553	1.18	3.92	1.71	5.63	761	15.1	70	3	306	67
240-	3	1918 May } June }	362	218	1.66	4.24	2.36	6.60	764	27.8	73	2	1640	357
330-	1	1919 Nov.	486	438	1.11	4.56	3.08	7.64	768	21.0	83	3	1142	248
340-	1	1919 Nov	728	423	1.70	4.09	2.01	6.10	750	21.8	73	5	292	64
No. 4														
(N. 35°-40°)														
160-	2	1915 Aug.	459	344	1.33	3.35	1.00	4.35	758	20.5	73	4	412	90
190-	2	1915 July	566	370	1.37	3.57	0.50	4.07	765	16.5	81	4	275	60
230-	1	1916 Sept.	600	465	1.29	3.68	2.61	6.29	768	16.8	83	4	488	106

Longitude	f	Date	n+	n-	$\frac{n+}{n-}$	R	γ°	q	Press.	Temp.	Humid.	W.V.	N	N _{1,2}
280-	3	1918 June 1919 Oct. }	162	219	0.74	3.79	9.06	12.85	767	21.2	71	4	31222	6787
290-	3	1919 Oct.	523	433	1.19	4.67	11.98	16.65	767	20.5	66	4	4942	1074
300-	1	1919 Oct.	339	368	0.92	2.84	10.85	13.69	761	22.5	84	6	8010	1741
310-	1	1919 Nov.	685	738	0.93	3.31	5.33	8.64	758	15.7	66	4	660	144
320-	3	1919 Nov.	725	619	1.17	4.04	4.00	8.01	754	17.0	73	3	524	114
330-	1	1919 Nov.		676		4.17	3.29	7.46	763	20.5	83	3		
(N. 40°-45°)														
150-	1	1919 Aug.	655	467	1.41	3.61	2.01	5.62	766	17.5	72	3	306	67
160-	1	1916 Aug.	495			3.42	2.80	6.22	758	17.3	87	2		
180-	1	1915 July	638	440	1.45	3.35	1.32	4.67	772	14.0	70	2	222	48
190-	1	1915 July	775	527	1.47	3.23	0.98	4.26	773	12.0	94	3	103	24
220-	3	1916 Sept.	672	468	1.44	3.28	6.87	10.15	770	17.7	81	4	1082	235
(N. 45°-50°)														
160-	5	1915 Aug.	623	435	1.44	3.24	4.71	7.95	757	14.7	93	2	756	165
180-	2	1916 Aug. Sept. }	568	422	1.35	2.37	15.25	17.62	765	10.9	93	2	4678	1017
190-	1	1915 July	572	467	1.23	3.46	0.47	3.93	768	13.0	90	5	193	42
(N. 50°-55°)														
170-	1	1915 Aug.	498	341	1.46	3.42	3.03	6.45	765	11.0	88	6	778	169
190-	2	1915 July	864	774	1.12	3.45	1.79	5.24	762	9.3	95	5	116	25

[illegible]

Longitude	<i>f</i>	Date	<i>n</i> +	<i>n</i> -	$\frac{n+}{n-}$	<i>R</i>	γ°	<i>q</i>	Press.	Temp.	Humid.	W.V.	<i>N</i>	<i>N</i> _{1,2}
340-	1	1919 Dec	655	514	1.28	4.06	0.94	5.30	762	23.8	70	5	262	57
350-	1	1919 Dec	718	496	1.45	4.20	0.59	4.79	700	25.1	77	5	171	37
No 6														
(S. 10°-15°)														
0-	3	1920 Mar	830	703	1.17	4.00	1.73	5.73	760	24.4	75	4	171	37
60-	1	1920 June	833	692	1.20	3.83	0.94	4.77	759	26.2	66	6	108	23
90-	1	1920 Aug.	732	637	1.15	4.18	0.54	4.72	760	27.8	77	0	148	32
160-	3	1915 Oct.	460	411	1.12	3.51	0.55	4.06	760	28.5	72	3	342	74
180-	1	1916 June	671	557	1.20	3.21	0.71	3.92	700	28.3	79	5	127	28
200-	1	1921 June	588	341	1.72	2.99	0.20	3.19	752	59.5	73	3	116	25
230-	1	1917 Jan	602	464	1.30	3.65	0.88	4.33	762	25.4	59	4	234	51
240-	2	1917 Jan.	533	437	1.22	3.10	1.02	4.12	764	24.9	67	7	249	54
260-	2	1918 Apr	520	405	1.28	3.57	0.00	3.57	761	26.8	67	8	196	43
270-	5	1918 Mar. } Apr } Feh. }	437 } 320 } 250 }		1.33 } 1.18 }	3.57 }	0.99 }	4.56 }	760 }	25.9 }	73 }	4 }	506 }	110 }
230-	2	1918 Mar	294	250	1.18	3.57	1.16	4.73	761	22.4	85	2	1232	268
340-	2	1919 Dec	685	604	1.13	4.03	0.31	4.34	763	24.1	67	5	148	32
350-	1	1920 Mar	746	563	1.31	3.82	5.29	9.11	760	24.2	78	3	660	144
S. 15°-20°														
0-	2	1920 Mar	827	766	1.08	4.03	6.74	10.77	753	23.0	76	4	707	154

80-	1	1920 Aug.	789	711	1.11	3.82	0.31	4.13	765	22.5	65	5	81	18
150-	2	1915 Oct.	583	466	1.26	3.39	2.04	5.43	763	26.8	72	5	376	82
180-	1	1916 June	429	567	1.60	3.01	0.00	3.01	760	26.4	86	2	234	51
220-	1	1917 Jan.	427	392	1.09	3.26	0.38	3.64	759	25.6	71	0	309	67
230-	2	1917 Jan.	558	396	1.41	4.14	3.03	7.17	762	26.5	71	4	756	165
240-	1	1917 Jan.	947			3.31	0.04	12.85	765	24.6	73	5		
260-	2	1918 Apr.	600	533	1.12	3.88	0.14	4.02	763	24.3	74	5	171	37
280-	1	1918 Feb.	291	232	1.26	3.88	0.00	3.88	762	27.5	68	2	829	180
320-	1	1920 Jan.	736	577	1.23	3.94	0.60	4.54	764	26.7	67	4	136	30
340-	1	1920 Apr.	654	529	1.24	3.51	0.82	4.53	764	25.4	63	4	171	37
350-	2	1920 Mar. } 1920 Apr.	796	692	1.15	4.24	3.14	7.38	763	24.4	73	4	342	74
No. 7														
S. 20°-25°)														
0-	1	1920 Mar.	712	607	1.17	3.56	1.31	4.87	758	22.2	75	4	171	37
80-	2	1920 Aug.	757	590	1.30	3.95	0.79	4.74	768	20.9	63	6	148	32
150-	3	1915 Oct.	412	279	1.48	2.82	3.38	9.20	763	24.7	72	2	2410	524
190-	2	1916 June	672	503	1.34	2.10	3.84	5.94	761	23.0	73	2	324	71
210-	2	1920 Dec.	596	484	1.33	3.66	0.24	3.90	764	28.0	71	4	171	37
230-	3	1916 Dec.	523	357	1.47	4.04	0.85	4.39	764	23.3	72	3	306	67
240-	2	1917 Jan	868	483	1.80	4.11	1.48	5.59	770	24.6	73	5	158	34
270-	3	1918 Feb.	417	309	1.74	4.12	0.41	4.53	761	24.1	61	2	547	119
330-	4	1920 Jan.	673	573	1.18	4.12	0.35	4.47	765	26.4	64	3	171	37

Longitude	<i>f</i>	Date	<i>n</i> +	<i>n</i> -	$\frac{n+}{n-}$	R	η°	<i>q</i>	Press.	Temp.	Humid.	W.V.	N	N _{1,2}
340- (S. 25°-30°)	2	1920 Apr.	566	562	1.11	3.83	0.66	4.49	764	16.0	67	1	262	57
0-	1	1920 Mar.	519	423	1.21	3.60	0.70	4.30	762	21.4	82	5	292	64
60-	2	1920 June	735	662	1.11	3.88	1.47	5.35	760	19.7	79	4	196	43
70-	3	1920 Aug.	973	812	1.20	3.57	1.69	5.26	771	17.0	74	4	90	20
80-	1	1920 Aug.	959	778	1.23	3.71	0.76	4.47	770	18.0	69	5	59	13
150-	3	1915 Oct.	537	499	1.10	2.86	2.11	4.97	766	21.9	61	4	357	78
190-	1	1916 June				2.14	2.20	4.34	758	21.5	79	5		
220-	5	1917 Jan. } 1920 Dec. }	605	426	1.42	3.44	0.37	3.81	765	25.3	78	3	153	34
250-	1	1917 Jan.	455	356	1.28	3.43	0.39	3.82	769	25.8	68	3	306	67
270-	4	1918 Feb. } Jan. }	495	385	1.29	3.43	0.31	3.74	766	21.4	67	5	278	60
280-	1	1918 Jan.					0.20		764	22.7	63	4		
330-	1	1920 Jan.	651	591	1.10	4.20	0.34	4.54	759	25.8	79	5	182	40
340-	1	1920 Apr.	669	589	1.14	4.20	0.47	4.67	757	24.0	77	5	182	40
No. 8														
S. (30°-35°)														
0-	2	1920 Mar.	581	341	1.70	4.17	0.45	4.62	767	20.5	62	2	278	60
30-	1	1920 May	768	581	1.32	3.26	0.19	3.45	766	17.5	65	4	66	14
70-	2	1920 Aug.	825	661	1.25	4.10	0.91	5.01	772	15.8	76	5	127	28

80-	1	1920 Aug.	903	662	1.26	3.94	0.38	4.2	772	15.3	90	5	73	16
90-	1	1916 Feb.	537			3.10	0.30	3.40	769	16.5	66	3		
150-	1	1915 Oct.	797	708	1.13	2.86	0.21	3.07	764	20.7	73	6	39	8
180-	2	1916 May				3.74	0.00	3.74	764	21.3	93	5		
220-	3	1917 Jan. 1920 Dec. }	546	344	1.59	3.53	0.31	3.84	762	20.4	83	3	222	48
240-	1	1916 Dec.	491	486	1.01	2.92	0.00	2.92	764	22.6	79	4	136	30
250-	2	1916 Dec				2.89	0.60	3.49	772	24.9	79	2		
260-	1	1918 Feb.	594	470	1.26	3.67	0.19	3.86	770	21.8	62	5	171	37
270-	3	1918 Feb.	493	421	1.17	3.67	0.43	4.10	766	21.3	63	4	292	64
280-	3	1918 Jan.	350	241	1.45	3.67	0.20	3.87	762	22.1	70	3	566	123
310-	3	1920 Jan.	519	464	1.12	3.67	2.37	6.04	759	21.4	60	3	357	78
320-	1	1920 Jan.	749	597	1.26	4.44	2.68	7.12	757	27.0	75	2	376	82
340-	2	1920 Apr.	507	556	0.91	4.05	0.55	4.60	751	21.4	81	5	342	74
No. 9														
(S. 35°-40°)														
10-	4	1920 Apr. }	422	600	0.70	3.66	3.09	6.75	773	18.0	82	4	1142	248
20-	1	1920 May }	994	745	1.27	3.27	0.68	3.95	756	12.8	71	4	39	8
30-	1	1920 May	745	792	0.94	3.65	0.40	4.05	762	14.6	70	6	90	20
80-	1	1920 Aug.	786	648	1.21	4.03	0.17	4.20	771	14.5	95	4	98	21
90-	5	1916 Feb.	623	509	1.23	3.30	0.35	3.65	766	16.0	77	4	127	23
110-	1	1920 Oct.	530	348	1.52	3.97	1.17	5.14	765	15.4	86	5	428	93
150-	1	1915 Oct.	515	451	1.14	2.81	15.66	18.47	754	18.0	87	1	32184	6997

Longitude	f	Date	n+	n-	$\frac{n+}{n-}$	R	η°	q	Press.	Temp.	Humid.	W.V.	N	N _{1,2}
160-	4	1915 Oct.	820	619	1.32	3.00	5.26	8.26	753	15.6	77	1	428	93
180-	1	1916 May	557	293	1.90	4.84	1.52	6.36	764	16.7	91	2	615	134
210-	2	1917 Jan. } 1920 Dec. }	555	473	1.17	3.31	0.31	3.62	761	15.2	86	4	171	37
220-	1	1917 Jan.	538	452	1.19	2.04	1.13	3.17	763	14.8	66	8	136	30
240-	3	1918 Feb.	804	635	1.26	2.96	0.20	3.16	770	20.7	65	3	46	10
270-	2	1918 Feb.	498	436	1.14	2.96	0.24	3.20	766	20.8	75	5	158	34
280-	1	1918 Jan. } 1917 {Feb. Dec. }	598	447	1.34	2.91	0.85	3.76	766	18.1	81	3	158	34
300-	3	1920 Feb. }	619	511	1.21	3.87	4.30	8.17	761	18.1	73	3	784	173
340-	1	1920 Apr.	604	617	1.08	4.18	0.33	4.51	768	16.3	66	1	171	37
350-	6	1920 Mar. } Apr. }	728	577	1.26	2.52	1.48	4.00	762	17.5	83	4	114	25
No. 10 (S. 40°-45°)														
80-	2	1916 Feb.	772	684	1.13	3.31	0.66	3.97	758	9.0	80	7	81	18
100-	1	1916 Feb.	673	609	1.10	3.35	2.12	5.47	763	13.5	86	6	562	57
130-	2	1916 Mar.	539	419	1.29	3.18	0.25	3.43	755	15.0	84	3	158	34
160-	2	1915 Oct. } 1916 Nov. }	727	654	1.11	3.01	8.37	11.38	749	12.8	83	6	1082	235
170-	6	1916 {Mar. Oct. }	870	550	1.58	3.59	4.14	7.73	761	12.8	88	3	324	70
180-	1	1920 Oct. } 1916 May }				1.31	1.83	3.19	759	16.7	88	6		
220-	3	1917 Feb.	475	354	1.34	2.63	0.64	3.32	764	13.9	76	5	207	45

280-	3	1918 Jan.	690	560	1.23	4.37	0.05	4.42	769	13.5	73	3	158	34
290-	2	1917 Dec.	528	600	0.98	4.37	40.44	44.81	763	17.8	63	5	39800	8652
310-	2	1920 Feb.	765	638	1.20	2.32	1.11	3.43	767	17.3	74	5	59	13
330-	1	1920 Mar.	550	381	1.44	4.53	0.11	4.64	773	11.8	86	4	309	67
340-	2	1920 Mar.	404	366	1.10	4.29	0.32	4.61	768	13.1	88	5	590	128
350-	1	1920 Mar.	479	454	1.05	4.45	0.32	4.77	762	14.7	89	1	428	93
No. 11 (8. 45°-50)														
40-	1	1916 Feb.	523	478	1.09	3.43	0.47	3.90	747	5.2	92	8	234	51
50-	1	1916 Feb.				3.39	0.55	3.94	760	7.2	84	4		
60-	1	1916 Feb.	491			3.55	0.19	3.74	759	6.3	82	4		
80-	1	1916 Feb.	759	675	1.20	3.23	0.08	3.31	750	4.6	82	8	59	13
100-	2	1916 Feb.	537	413	1.30	3.39	0.59	3.98	754	6.6	90	3	234	51
130-	2	1916 Mar.	767	524	1.31	3.81	0.42	4.23	747	10.6	85	6	108	23
160-	4	1916 Mar. 1920 Oct.	794	639	1.24	7.57	0.73	8.30	766	10.5	81	5	529	115
170-	4	1915 {Nov. Dec. 1916 Mar. 1920 Oct.	542	420	1.29	3.73	5.14	8.87	759	10.3	90	4	936	209
180-	1	1915 Oct.	753	648	1.16	3.07	0.98	4.05	749	12.0	78	6	98	21
230-	2	1917 Feb.	582	454	1.23	2.65	1.48	4.13	761	10.6	77	6	207	45
240-	2	1917 Feb.	560	392	1.29	3.12	1.04	4.16	763	9.0	82	7	292	64
270-	1	1918 Jan.	762	524	1.45	3.74	0.00	3.74	753	9.0	71	6	81	18
290-	1	1917 Dec.	531	624	0.85	3.74	20.97	24.71	751	15.5	62	5	10547	2293

Longitude	<i>f</i>	Date	<i>n</i> +	<i>n</i> -	$\frac{n+}{n-}$	<i>R</i>	τ°	<i>q</i>	Press.	Temp.	Humid.	W.V.	<i>N</i>	<i>N</i> _{1,2}
330- No. 12 (S. 50°-55°)	1	1920 Mar.	568	505	1.16	4.35	0.49	4.84	770	10.7	87	5	278	60
0-	3	1916 Jan.	536	516	1.04	3.29	0.51	3.80	752	2.5	80	5	196	43
10-	1	1916 Jan.	618	537	1.15	3.50	0.26	3.76	741	2.5	79	5	136	30
20-	2	1916 Jan.	504	447	1.13	3.50	0.27	3.77	730	2.0	87	4	234	51
30-	1	1916 Jan.	471	413	1.14	3.67	0.30	3.97	737	6.4	85	5	309	67
40-	1	1916 Jan.	418	367	1.14	3.41	5.62	9.03	741	3.5	84	7	1797	391
70-	3	1916 Feb.	511	415	1.23	3.73	0.40	4.13	748	3.7	88	7	278	60
110-	2	1916 Mar.	497	406	1.22	3.42	0.56	3.98	755	4.1	86	5	278	60
150-	1	1916 Mar.	743	635	1.17	3.14	0.38	3.52	750	7.7	78	4	73	16
160-	1	1916 Mar.	614	564	1.09	3.57	0.23	3.85	747	10.0	82	7	148	32
180-	2	1915 Dec.	743	631	1.19	3.46	0.66	4.12	752	8.8	88	3	108	23
190-	1	1915 Dec.	676	535	1.26	3.82	0.09	3.91	746	6.8	78	5	108	23
270-	1	1918 Jan.	426	332	1.28	3.88	0.71	4.59	759	8.0	94	6	529	115
290-	3	1917 Feb. Dec. }	724	596	1.22	3.96	4.63	8.59	746	8.4	68	5	615	134
310-	1	1916 Jan.	792	765	1.04	3.56	0.58	4.14	754	4.7	93	3	81	18
320-	1	1916 Jan.	664	602	1.10	3.75	0.51	4.26	740	4.0	85	6	148	32
340-	2	1916 Jan.	337	247	1.36	3.46	0.83	4.29	744	3.5	95	4	784	170

[illegible]

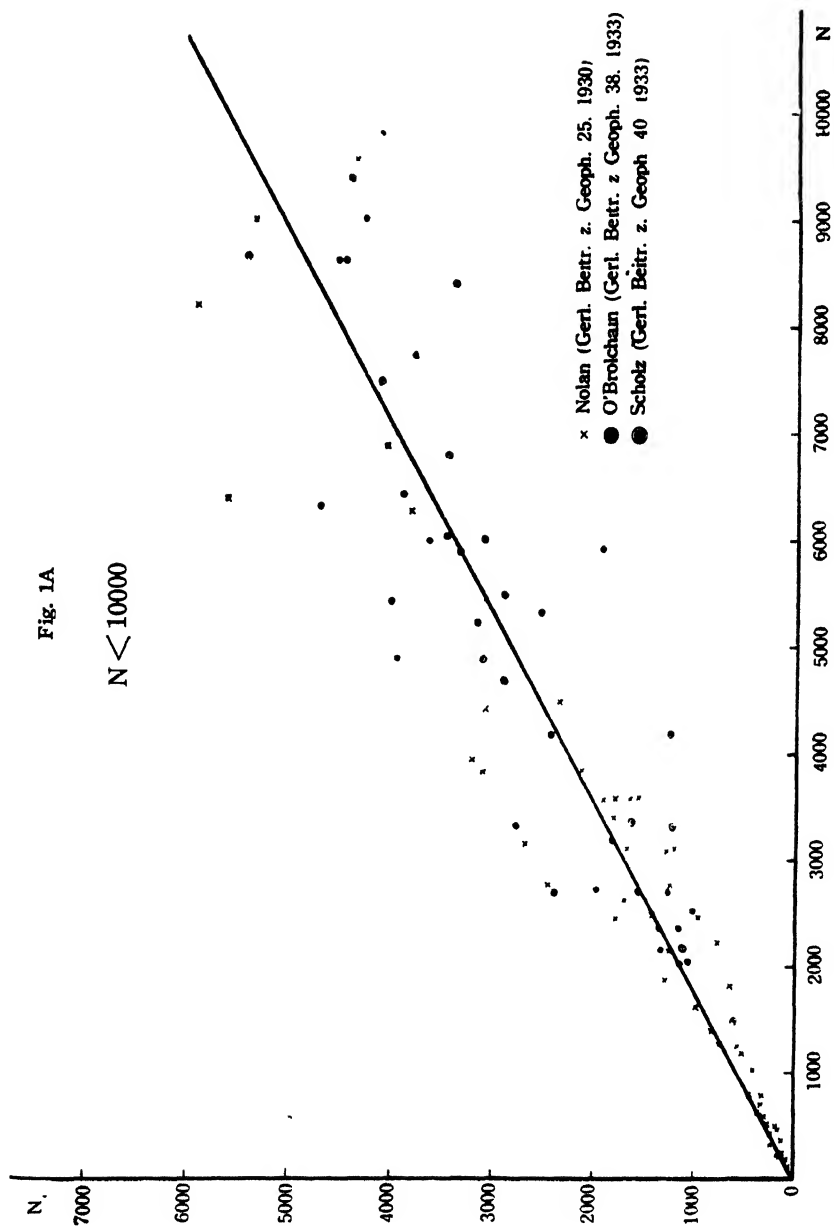
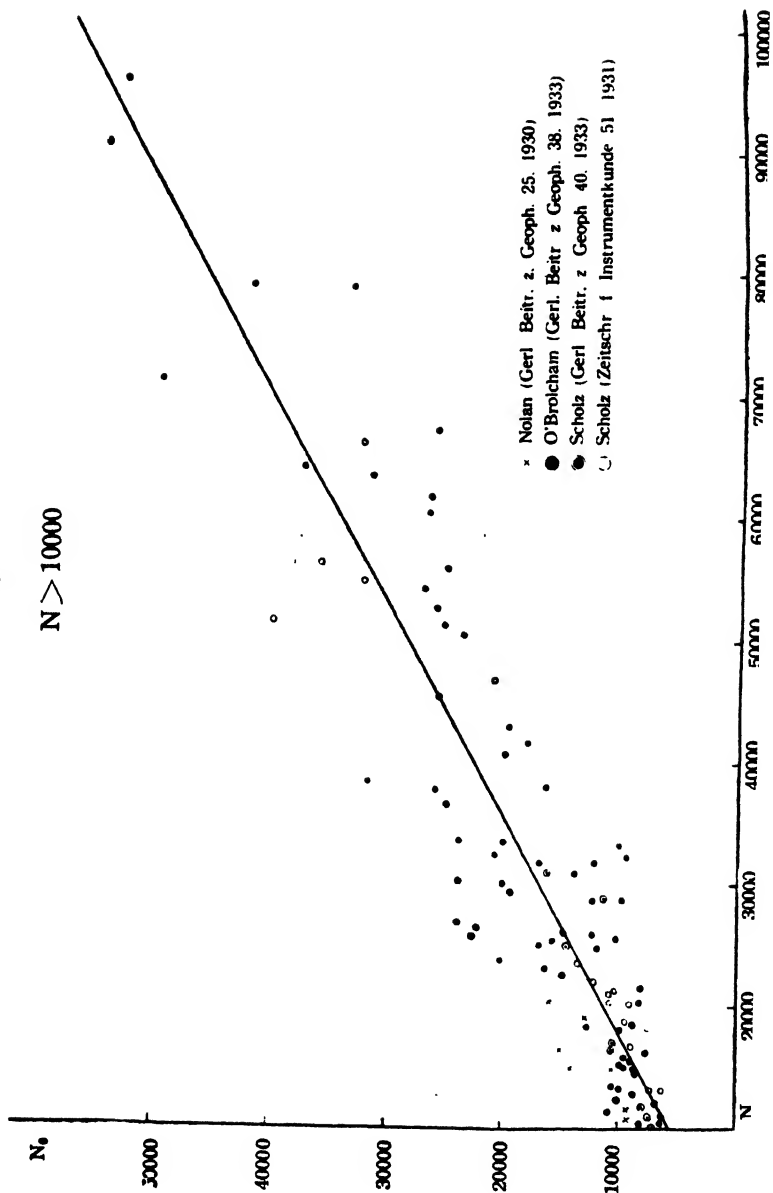
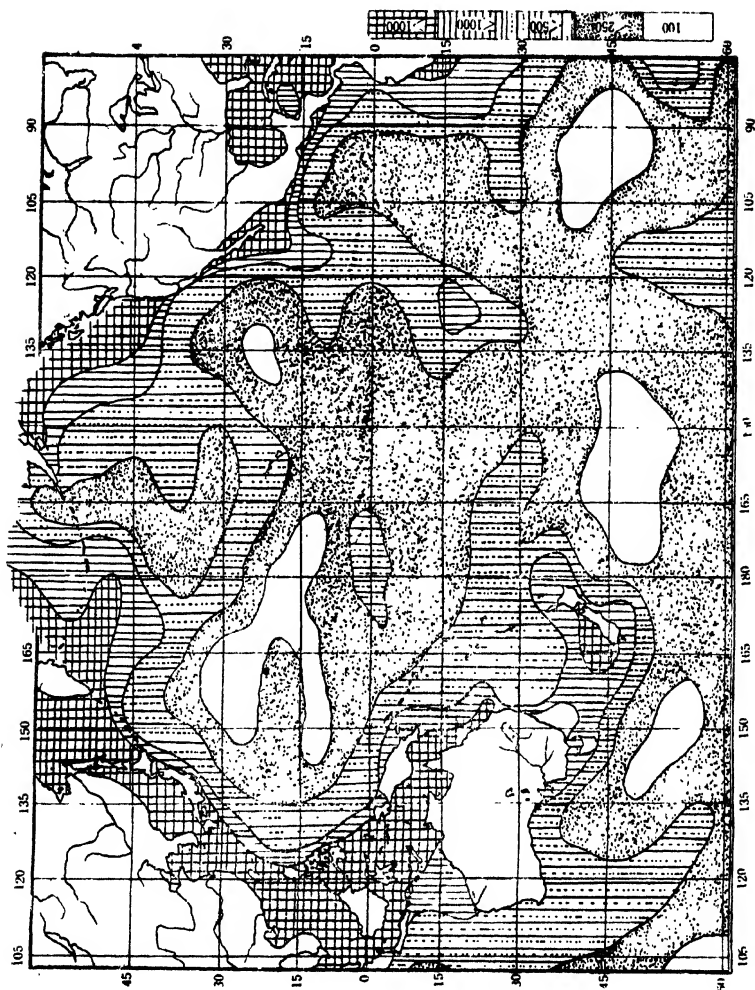
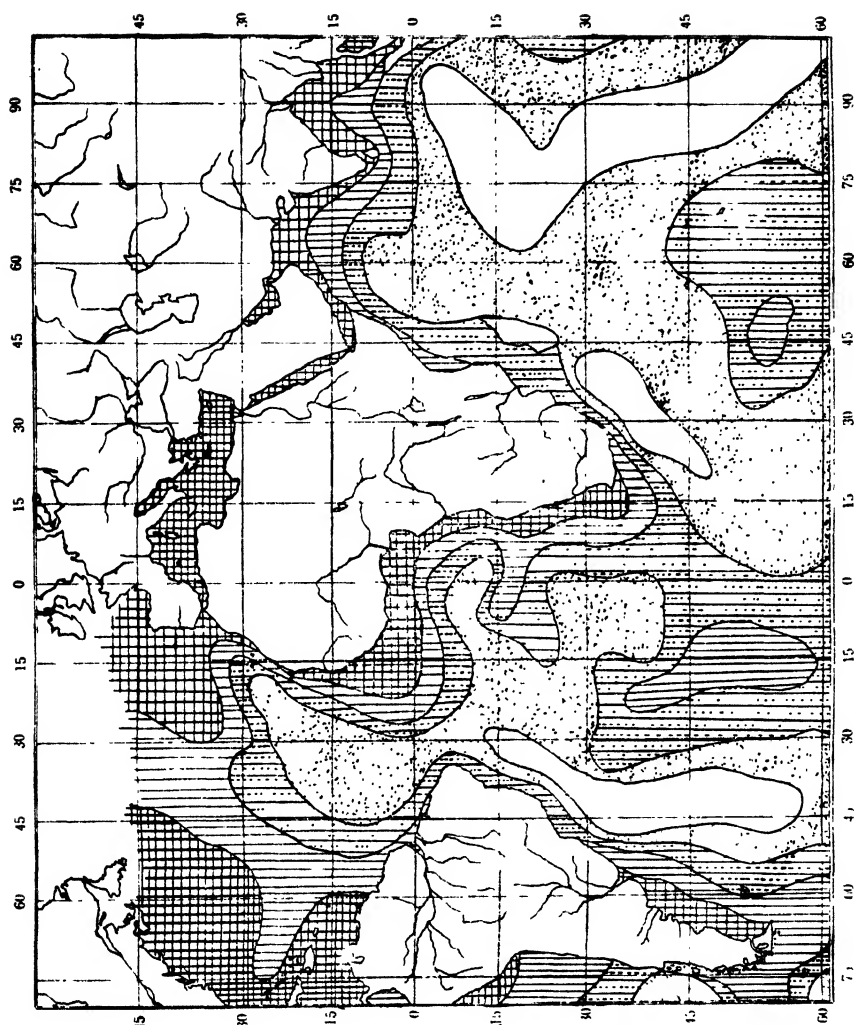


Fig. 1B







ATMOSPHERIC ELECTRICAL CONDITION AND METEOROLOGICAL ELEMENTS

Katsuyoshi, SHIRATORI

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The influence of the meteorological conditions upon the atmospheric electrical conductivity still remains one of the most interesting of problems, and in this paper we have tried to find out statistically, how the conductivity is affected by the meteorological elements, by treating the numerous results of the hourly-observations of the conductivity which were taken during 1931 to 1932 at Taihoku. The method of observation and the boundary locality where this experiment was undertaken has already been described in a previous paper.⁽¹⁾ The six elements (temperature, pressure, relative humidity, cloudiness, wind velocity and wind direction) are taken from the meteorological conditions, special care being paid relative to thunder-showers, typhoons and monsoons.

I. Average Deviation and Variance of Conductivity.

The annual means or the hourly means of the conductivity must be considered in connection with their mean square deviations and their variances which are the ratios of the standard deviations to their mean values; because if such mean square deviations or variances are large, then it is known that the individual data are distributed for a wide-range around the means, therefore these two terms are very important as they show a measure of the preciseness of the degree represented by the mean value and also a measure of

fluctuation or unsteadiness of the factors. The table "IA" is the variance of the monthly means and the table "IB" that of the hourly means of each season* of the atmospheric electrical condition in which tables "A" is the mean square deviations, " δ " is the variance, and " n " is the no. of the individuals used and also the means of the corresponding meteorological elements are added.

For the total conductivity (λ) the average deviation is about 2.5×10^{-4} e.s.u. in winter and 1.4×10^{-4} e.s.u. in summer; the maximum 2.8×10^{-4} e.s.u. of December, the minimum 1.2×10^{-4} e.s.u. of July and the variance is almost less than 0.30 in winter and more than 0.35 in summer, the maximum 0.64 of April, the minimum 0.27 of January. Hence we can say that the condition of the atmospheric conductivity is relatively much more unsteady in summer than in winter, notwithstanding that the conductivity is twice as less in summer. Such unsteadiness of the conductivity in summer must be due to the influences of thunderstorms and heavy showers, and also due to the unstability of wind velocity in summer, which variance is very large during April to September. The negative polar conductivity ($\lambda-$) has the average deviation amounted from 1.2×10^{-4} e.s.u. to 1.5×10^{-4} e.s.u. in winter and from 0.6×10^{-4} e.s.u. to 0.9×10^{-4} e.s.u. in summer, the maximum 1.5×10^{-4} e.s.u. of November, the minimum 0.6×10^{-4} e.s.u. of July, and also has the variance of order about 0.40 in summer and below 0.35 in winter, the maximum 0.61 of April, the minimum 0.31 of January. The deviation of the positive polar conductivity ($\lambda+$) is 0.7×10^{-4} e.s.u. in summer and about 1.5×10^{-4} e.s.u. in winter, the maximum 1.6×10^{-4} e.s.u. of December; its variance is about 0.3 in winter and about 0.4 in summer.

In winter the variance of $\lambda+$ is much less than that of $\lambda-$ in spite of the fact that in summer both variances of $\lambda+$ and $\lambda-$ become nearly of the same order and the mean deviation is more un-uniform for $\lambda-$ than for $\lambda+$; hence it may be concluded that the negative

* The four selected seasons are January-March, April-June, July-September and October-December the same as in my previous paper⁽¹⁾.

polar conductivity is more fluctuable and more unsteady than the positive, and that the positive polar conductivity is more steady in winter than in summer.

During summer June and July are minimum for the mean deviation and have comparatively smaller variances, as seen from the table $\lambda+$ in June and both $\lambda+$ and $\lambda-$ in July are very steady. During winter January is the minimum for variance and most stable for the electrical condition of air. It is noted that these steady months have just the same proportion of maximum or minimum in the annual oscillation of the atmospheric conductivity.

April has the largest variance of the year in which month the winter type is gradually alternated by the summer type, both types reoccurring simultaneously. From September to October the deviation and the variance show a distinct gap in amount, and this change also corresponds sharply to the beginning of the winter monsoon weather instead of the summer type.

The variance or deviation of the ratio (q_λ) of both polar conductivities is small during from October to January and relatively large in February, March and June.

Concerning the meteorological elements the relative humidity shows a large variance in March and April, corresponding to the unsteadiness of λ in April, and the wind velocity has a large variance in summer, the variance of cloudiness being particularly small in February, May and June. As to the pressure the variance is relatively large in August, September, December and March; the temperature being large in winter but very small for summer.

These deviations or variances of the meteorological factors account for so many complicated results in comparison with those of the atmospheric electrical conditions that it is very difficult to find some certain significant association between purely single factors without eliminating the influence of the others.

In view of the diurnal oscillation we can account for some certain factors as to the deviation and variance, from the table "IB". It is clear that the deviation by unit of 10^{-4} e.s.u. for λ , $\lambda-$,

λ_+ are respectively about 2.5, 1.3, 1.4, in the first season; 1.5, 0.8, 0.9, in the second season; 1.3, 0.7, 0.7, in the third season and 2.5, 1.2, 1.4, in the fourth season; therefore the value of the winter type is much larger than the summer type, and also the values of λ_+ are larger than those of λ_- in winter, but oppositely both become nearly same in summer; and that the variances of λ , λ_- and λ_+ are respectively about 0.30, 0.34, 0.30, in the first season, 0.39, 0.45, 0.41 in the second season, 0.36 0.39, 0.38, in the third season and 0.29, 0.31, 0.32 in the fourth season; hence the variances of the summer type are so much greater than the winter type that the electrical conditions are relatively much more fluctuable in summer and from the given results of the first and third season, the variance for λ_- seems larger than that for λ_+ in the winter type and contrary to in summer.

The smallest deviation or variance in the winter type is near the 12 h. when the minimum of λ in the diurnal oscillation occurs, though in the summer type such point is not so sharply clear. The deviation and variance of the meteorological factors corresponding to the diurnal oscillation of the atmospheric electricity are summarized as such: Those for relative humidity, cloudiness and temperature are larger in winter and those for wind velocity are larger in summer, likewise those for pressure being somewhat large in the first and third season.

TABLE 1A.

	γ		$\gamma -$		$\gamma \perp$		q_1		Relative Humidity		
	Δ	δ	Δ	δ	Δ	δ	Δ	δ	mean	Δ	δ
January	2.6	0.27	1.4	0.31	1.5	0.29	0.30	0.25	72	14	0.19
February	2.4	0.29	1.3	0.35	1.3	0.29	0.45	0.35	82	12	0.15
March	2.4	0.35	1.2	0.40	1.3	0.34	0.45	0.32	69	16	0.23
April	2.5	0.64	1.1	0.61	1.5	0.72	0.30	0.26	73	16	0.22
May	1.4	0.37	0.8	0.46	0.7	0.34	0.35	0.27	77	10	0.13
June	1.3	0.29	0.7	0.35	0.7	0.28	0.45	0.34	78	12	0.15
July	1.2	0.34	0.6	0.33	0.7	0.40	0.25	0.25	65	10	0.15
August	1.5	0.41	0.9	0.45	0.7	0.41	0.30	0.33	69	12	0.17
September	1.3	0.37	0.8	0.45	0.7	0.41	0.25	0.26	71	12	1.17
October	2.3	0.28	1.2	0.31	1.3	0.30	0.25	0.22	80	12	0.15
November	2.4	0.28	1.5	0.36	1.4	0.31	0.25	0.23	74	10	0.14
December	2.8	0.32	1.4	0.34	1.6	0.34	0.30	0.26	78	12	0.15

	Wind Velocity			Cloudiness			Pressure			Temperature			n
	mean	Δ	δ	mean	Δ	δ	mean -700	Δ	δ	mean	Δ	δ	
January	3.1	1.5	0.48	7	4	0.57	67.8	2.5	0.003	11.4	4.0	0.35	149
February	2.5	1.5	0.60	9	2	0.22	66.0	3.0	0.004	14.5	4.5	0.31	148
March	2.5	1.5	0.60	7	4	0.57	63.7	3.5	0.005	18.2	4.0	0.22	156
April	2.6	2.0	0.77	8	4	0.50	60.7	3.0	0.004	22.0	4.5	0.20	107
May	2.0	1.5	0.75	8	2	0.25	58.0	2.5	0.003	28.1	2.5	0.09	133
June	1.9	1.5	0.79	9	2	0.22	55.1	2.5	0.003	23.7	3.0	0.10	195
July	1.6	1.5	0.94	7	4	0.57	55.3	3.0	0.004	31.1	2.0	0.05	343
August	2.3	2.0	0.87	7	4	0.57	52.3	3.5	0.005	30.3	2.0	0.07	143
September	2.1	1.5	0.71	7	4	0.57	56.9	3.5	0.005	29.1	3.0	0.10	170
October	3.0	1.5	0.50	8	4	0.50	61.9	3.0	0.004	22.8	2.5	0.11	148
November	2.9	1.5	0.52	7	4	0.57	62.5	3.0	0.004	24.3	4.0	0.16	148
December	2.4	1.5	0.63	8	4	0.50	65.6	3.5	0.005	19.5	4.5	0.23	133

TABLE IB.

Season	Time	γ				$\gamma +$				q_1				Relative Humidity		
		Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	mean	Δ	δ
II	10 h.	2.5	0.31	1.3	0.36	1.4	0.32	0.25	0.20	0.25	0.20	76	12	76	12	0.16
	11	2.5	0.32	1.3	0.36	1.3	0.31	0.35	0.28	0.35	0.28	74	14	74	14	0.19
	12	1.7	0.23	1.0	0.31	1.0	0.24	0.55	0.39	0.55	0.39	72	16	72	16	0.22
	13	2.4	0.28	1.2	0.30	1.5	0.33	0.35	0.29	0.35	0.29	71	16	71	16	0.23
	14	2.7	0.31	1.5	0.33	1.5	0.31	0.45	0.35	0.45	0.35	72	14	72	14	0.19
	15	2.8	0.30	1.4	0.34	1.6	0.32	0.40	0.31	0.40	0.31	73	14	73	14	0.19
	16	2.4	0.27	1.2	0.30	1.3	0.25	0.35	0.27	0.35	0.27	76	14	76	14	0.18
	17	2.6	0.36	1.2	0.40	1.4	0.34	0.35	0.24	0.35	0.24	78	12	78	12	0.15
	9	1.3	0.30	0.7	0.36	0.8	0.31	0.30	0.24	0.30	0.24	81	10	81	10	0.12
	10	1.5	0.37	0.7	0.38	0.9	0.41	0.30	0.25	0.30	0.25	76	12	76	12	0.16
	11	1.6	0.43	0.8	0.47	0.9	0.44	0.45	0.36	0.45	0.36	74	14	74	14	0.19
	12	1.8	0.47	0.9	0.55	1.1	0.50	1.00	0.72	1.00	0.72	73	12	73	12	0.16
	13															
	14	2.0	0.43	1.2	0.53	1.0	0.43	0.35	0.32	0.35	0.32	76	12	76	12	0.16
	15	1.8	0.43	0.8	0.44	1.1	0.46	0.55	0.40	0.55	0.40	77	12	77	12	0.16
	16	1.6	0.37	0.8	0.41	0.8	0.33	0.40	0.30	0.40	0.30	79	12	79	12	0.15
	17	1.4	0.35	0.8	0.46	0.8	0.35	0.65	0.44	0.65	0.44	82	12	82	12	0.15

Season	Time	Wind Velocity			Cloudiness			Pressure			Temperature			n
		mean	Δ	δ	mean	Δ	δ	mean —700	Δ	δ	mean	Δ	δ	
I	10 h.	2.3	1.5	0.65	7	4	0.57	67.1	3.0	0.004	16.6	3.5	0.21	53
	11	2.5	1.5	0.60	7	4	0.57	66.7	3.0	0.004	16.8	4.0	0.24	56
	12	2.6	1.5	0.58	7	4	0.57	66.2	3.0	0.004	17.4	4.5	0.26	69
	13	3.0	1.5	0.50	7	4	0.57	65.7	2.5	0.003	18.3	4.5	0.25	37
	14	2.9	1.5	0.69	8	4	0.50	65.2	3.0	0.004	17.3	4.5	0.26	64
	15	2.9	1.5	0.52	8	4	0.50	65.1	3.0	0.004	16.8	4.5	0.27	64
	16	2.8	1.5	0.54	8	4	0.50	65.2	3.0	0.004	16.3	4.0	0.25	61
	17	2.4	1.5	0.63	8	4	0.50	64.9	3.5	0.005	16.3	4.0	0.25	40
	9	1.7	1.5	0.89	9	2	0.22	55.8	2.5	0.003	27.8	2.5	0.09	27
	10	1.8	1.5	0.83	8	2	0.25	58.4	2.5	0.003	26.7	3.0	0.11	61
	11	1.9	1.5	0.79	8	2	0.25	58.4	2.5	0.003	26.8	3.5	0.13	63
	12	2.4	1.5	0.63	9	2	0.22	58.2	2.5	0.003	27.4	4.0	0.15	55
	13													
	14	2.4	1.5	0.63	9	2	0.22	57.1	2.5	0.003	27.2	3.5	0.13	69
	15	2.5	1.5	0.60	9	2	0.22	56.7	2.5	0.003	26.9	3.5	0.13	65
	16	2.3	1.5	0.65	9	2	0.22	56.8	2.5	0.004	26.2	3.5	0.13	60
	17	1.8	1.5	0.83	9	2	0.22	56.5	2.0	0.003	26.3	3.0	0.11	35
II	9	1.7	1.5	0.89	9	2	0.22	55.8	2.5	0.003	27.8	2.5	0.09	27
	10	1.8	1.5	0.83	8	2	0.25	58.4	2.5	0.003	26.7	3.0	0.11	61
	11	1.9	1.5	0.79	8	2	0.25	58.4	2.5	0.003	26.8	3.5	0.13	63
	12	2.4	1.5	0.63	9	2	0.22	58.2	2.5	0.003	27.4	4.0	0.15	55
	13													
	14	2.4	1.5	0.63	9	2	0.22	57.1	2.5	0.003	27.2	3.5	0.13	69
	15	2.5	1.5	0.60	9	2	0.22	56.7	2.5	0.003	26.9	3.5	0.13	65
	16	2.3	1.5	0.65	9	2	0.22	56.8	2.5	0.004	26.2	3.5	0.13	60
	17	1.8	1.5	0.83	9	2	0.22	56.5	2.0	0.003	26.3	3.0	0.11	35
	9	1.7	1.5	0.89	9	2	0.22	55.8	2.5	0.003	27.8	2.5	0.09	27
	10	1.8	1.5	0.83	8	2	0.25	58.4	2.5	0.003	26.7	3.0	0.11	61
	11	1.9	1.5	0.79	8	2	0.25	58.4	2.5	0.003	26.8	3.5	0.13	63
	12	2.4	1.5	0.63	9	2	0.22	58.2	2.5	0.003	27.4	4.0	0.15	55
	13													
	14	2.4	1.5	0.63	9	2	0.22	57.1	2.5	0.003	27.2	3.5	0.13	69
	15	2.5	1.5	0.60	9	2	0.22	56.7	2.5	0.003	26.9	3.5	0.13	65
	16	2.3	1.5	0.65	9	2	0.22	56.8	2.5	0.004	26.2	3.5	0.13	60
	17	1.8	1.5	0.83	9	2	0.22	56.5	2.0	0.003	26.3	3.0	0.11	35

Season	Time	λ		$\lambda -$		$\lambda +$		q_{λ}		Relative Humidity		
		Δ	δ	Δ	δ	Δ	δ	Δ	δ	mean	Δ	δ
III	9 h.	1.4	0.40	0.8	0.45	0.8	0.46	0.25	0.25	69	10	0.14
	10	1.3	0.37	0.7	0.39	0.6	0.34	0.15	0.15	65	10	0.15
	11	1.3	0.39	0.7	0.40	0.6	0.37	0.25	0.26	63	10	0.16
	12	1.2	0.37	0.7	0.42	0.6	0.38	0.25	0.25	64	10	0.16
	13	1.2	0.36	0.7	0.41	0.6	0.37	0.35	0.35	67	10	0.15
	14	1.2	0.33	0.8	0.42	0.7	0.40	0.25	0.27	68	12	0.18
	15	1.3	0.35	0.7	0.35	0.7	0.39	0.25	0.27	69	10	0.14
	16	1.3	0.33	0.8	0.39	0.7	0.35	0.30	0.31	71	12	0.17
	17	1.3	0.34	0.6	0.32	0.7	0.37	0.25	0.25	74	10	0.14
IV	10	2.2	0.29	1.1	0.30	1.3	0.32	0.25	0.22	78	14	0.18
	11	2.1	0.28	1.0	0.28	1.2	0.30	0.20	0.18	77	12	0.16
	12	1.9	0.25	0.9	0.27	1.2	0.29	0.30	0.24	77	12	0.16
	13	2.6	0.33	1.2	0.34	1.5	0.35	0.25	0.21	76	12	0.16
	14	2.7	0.29	1.4	0.31	1.5	0.31	0.25	0.23	77	12	0.16
	15	2.9	0.29	1.5	0.31	1.6	0.31	0.25	0.23	78	10	0.13
	16	2.8	0.29	1.4	0.31	1.6	0.32	0.25	0.23	79	10	0.13
	17	2.6	0.32	1.4	0.37	1.5	0.36	0.30	0.27	82	8	0.10

Season	Time	Wind Velocity			Cloudiness			Pressure			Temperature			n
		mean	Δ	δ	mean	Δ	δ	mean —700	Δ	δ	mean	Δ	δ	
III	9 h.	1.2	2.0	1.67	5	4	0.80	55.4	3.5	0.005	29.5	2.0	0.07	62
	10	1.4	1.5	1.07	6	4	0.67	55.3	3.5	0.005	30.4	2.0	0.07	91
	11	1.6	2.0	1.25	6	4	0.67	55.2	3.5	0.005	31.1	2.0	0.06	90
	12	1.8	1.5	0.83	7	4	0.57	55.3	3.5	0.005	31.3	2.0	0.06	75
	13	2.4	1.5	0.63	7	4	0.57	54.2	4.0	0.005	30.5	3.0	0.10	41
	14	2.2	1.5	0.68	8	4	0.50	54.4	3.5	0.005	30.9	2.5	0.08	100
	15	2.1	1.5	0.71	8	2	0.25	54.8	3.5	0.005	30.6	2.5	0.08	95
	16	1.8	1.5	0.83	8	2	0.25	55.5	3.0	0.004	29.5	2.5	0.08	68
	17	1.7	1.5	0.88	8	4	0.50	56.4	3.5	0.005	28.8	2.5	0.03	34
IV	10	2.3	1.5	0.65	8	4	0.50	64.3	3.0	0.004	21.5	3.5	0.16	53
	11	2.7	1.5	0.56	8	2	0.25	64.3	3.0	0.004	21.8	4.0	0.18	64
	12	2.8	1.5	0.54	8	4	0.50	63.6	3.0	0.004	22.5	4.0	0.18	61
	13	3.2	1.5	0.47	6	4	0.66	62.2	3.0	0.004	23.7	3.5	0.15	19
	14	3.1	1.5	0.48	8	4	0.50	62.9	3.0	0.004	22.1	4.0	0.18	62
	15	3.0	1.5	0.50	8	4	0.50	62.7	3.0	0.004	22.0	4.0	0.18	62
	16	2.7	1.5	0.60	8	4	0.50	62.6	3.0	0.004	21.6	3.5	0.16	61
	17	2.5	1.5	0.60	7	4	0.57	62.4	3.0	0.004	21.7	3.0	0.14	37

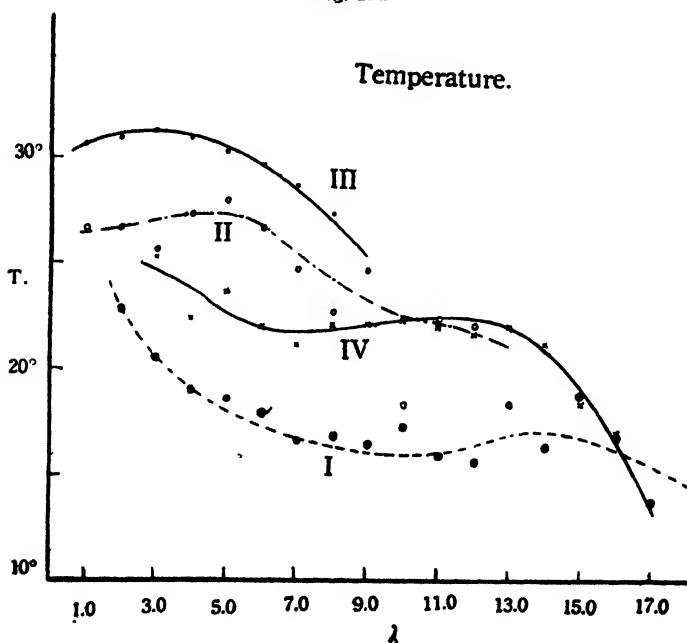
2. Groups of Conductivity.

Individual observations have been grouped according to the value of the conductivity and the means of the associated values of the meteorological elements for such each group were determined. The frequency distributions of such groups of the conductivity appear to give nearly the same form like a probability curve and have one maximum.

The results thus obtained are in the tables II.

In the first season the distribution of the total conductivity (λ) is ranged from $1. \times 10^{-4}$ e.s.u. to 16×10^{-4} e.s.u. and the maximum frequency is of the value of 8×10^{-4} e.s.u. In the second season the range is extended to 12×10^{-4} e.s.u., having the maximum frequency at $4. \times 10^{-4}$ e.s.u. and in the third season it covers up to 7×10^{-4} e.s.u., the maximum frequency being $3. \times 10^{-4}$ e.s.u. In the fourth season it ranges from $3. \times 10^{-4}$ e.s.u. to 16×10^{-4} e.s.u. showing the maximum frequency at $8. \times 10^{-4}$ e.s.u.

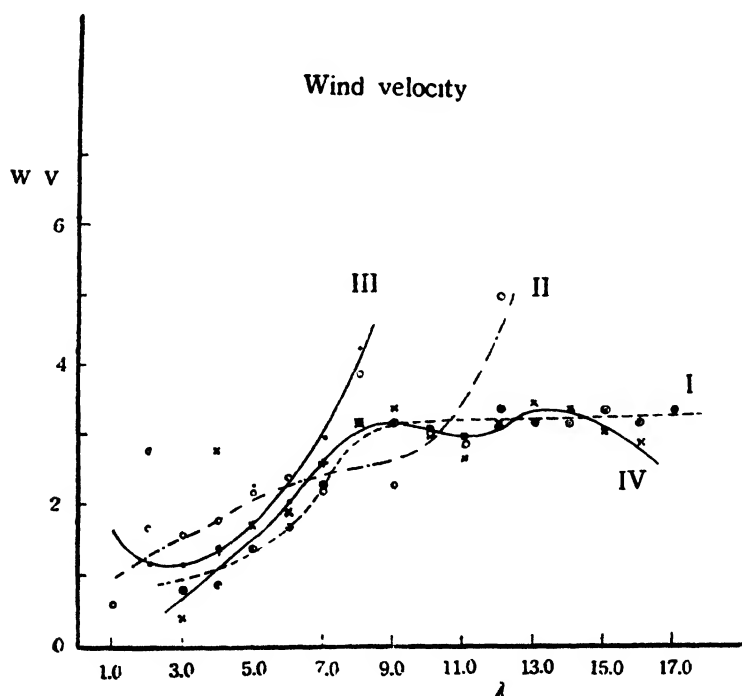
Fig. 2A.



The Fig. 2A shows graphically the relation of λ to the mean temperature for each corresponding group. On the whole the group of larger λ corresponds generally to a lower value of the mean temperature. Especially in winter, such a tendency is very regular, even though as clearly seen from the curve for summer type increasing λ corresponds to an increase of temperature within the limit of a certain value but beyond this limit, then it corresponds to a decrease of temperature, therefore the relation is not linear.

Dealing with the mean value of relative humidity for each group of λ it appears to have no regular association, roughly speaking. In the summer season the larger values of relative humidity correspond to groups of larger λ , and in winter there is no regularity at all, on the other hand an inversely larger humidity, tends to a correspondingly rather smaller conductivity.

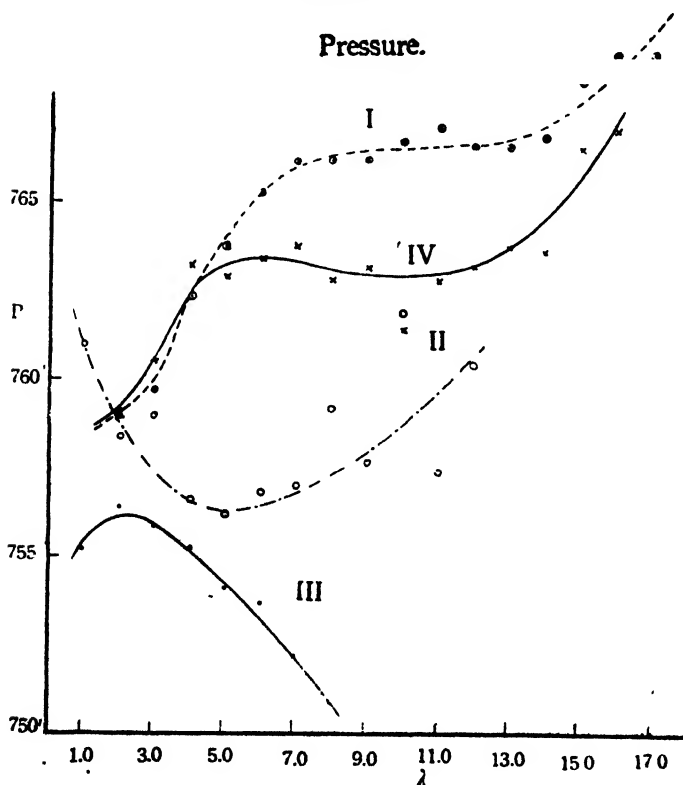
Fig. 2B.



Regarding the association between the mean amount of cloud and the groups of λ there cannot be found any regularity apparently, and only in the third season does there seem to be a slight tendency of increasing λ with cloudiness through the influence of thunder and typhoons but in the fourth season the large group of λ associates with a comparatively smaller amount of cloudiness generally.

In respect to the means of wind velocity for such groups, the larger velocity corresponds to the larger group of λ . In the summer season the rate of increase of the wind velocity is due to the increase of λ -group and is comparatively large, in spite of the fact that in winter the group of λ above 12×10^{-4} e.s.u. this being the mean of wind velocity, becomes constant as seen in Fig. 2B.

Fig. 2C.



The distribution of the means of pressure for λ -groups is shown in the Fig. 2C. It may be noted that in winter the pressure is increased rapidly with an increase of λ -groups until 5×10^{-4} e.s.u., and then it becomes constant even when λ increases up to the group of 11×10^{-4} e.s.u., and again it increases rapidly with the group of the far larger λ .

As for the summer type such a relationship of pressure for λ -groups is quite different. In the second season the mean of pressure is decreased until the λ -group becomes into 5×10^{-4} e.s.u., above this group the mean pressure tends to increase with λ . In the third season it can be seen more clearly that the mean pressure decreases with an increase of λ -group. This different characteristic might be due to the influence of the low of a typhoon, for this increase of λ was observed during the low of a typhoon,—and this typical summer character seems to be general in the third season.

In summer the pressure and wind velocity are inversely related, the pressure is lower, the wind velocity is larger, and therefore the conductivity λ associates with the lower value of pressure and the larger value of wind velocity; but in winter the pressure and wind velocity are nearly in parallel relation and thus λ increases with them. Such characteristicity will be more clearly tested by the individual correlations, later.

Both polar conductivities, positive and negative, have a wider range of distribution in winter than in summer and in general the λ_+ of the maximum frequency is larger than the λ_- of the same except in the third season (in which season both conductivities are nearly equal.) In the first season the decreasing of $\lambda_{+,-}$ -group is almost associated with the decreasing of the means of temperature and pressure, and with the increasing of wind velocity; and larger $\lambda_{+,-}$ -group tends to correspond to a larger amount of cloud. Relative humidity does not show any such regular distribution.

In the second season no significant tendency of such grouped mean of meteorological elements, except wind velocity which seemed somewhat remarkable; and only for the larger group of $\lambda_{+,-}$ is the

mean temperature comparatively low and humidity or cloudiness large, For the increasing of λ_{+-} -group, the mean wind velocity becomes larger.

The third season gives the characteristic summer type and smaller value of the mean temperature and the pressure or larger value of mean wind velocity, humidity and cloudiness, correspond to the larger group of λ_{+-} and the influence of thunderstorm and typhoons being very large as described before.

In the fourth season both polar conductivities associate very regularly with meteorological elements; the larger group of λ_{+-} associates with a lower mean temperature, and the larger means of humidity or cloudiness correspond to the comparatively lower group of λ_{+-} , and the wind velocity or pressure becomes larger according to the increases of λ_{+-} ; hence the effect of monsoon may be said to be fairly powerful.

TABLE II.
No. 1 Group of λ
I Season (1-2-3)

λ (10^{-4} e.s.u.)	n	Temp.	Hum.	Wind Vel.	Cloud	Press.
2.0>	1	22.9	96	2.8	10	59.2
3.0	7	20.5	73	0.8	7	59.7
4.0	33	19.0	70	0.9	8	62.4
5.0	40	18.7	72	1.4	9	63.8
6.0	30	18.1	70	1.7	7	65.3
7.0	58	16.6	74	2.3	8	66.2
8.0	56	16.8	74	3.2	8	66.3
9.0	73	16.5	75	3.2	8	66.2
10.0	51	17.0	74	3.1	7	66.7
11.0	46	16.0	94	3.0	8	67.1
12.0	38	15.7	77	3.4	8	66.6
13.0	14	18.6	76	3.2	8	66.6
14.0	13	16.4	74	3.2	8	66.7
15.0	3	18.7	73	3.4	8	68.5
16.0	3	16.9	66	3.2	9	69.2
17.0>	2	13.8	75	3.4	7	69.2

II Season (4-5-6)

λ (10^{-4})	n	Temp.	Hum.	Wind Vel.	Cloud	Press.
1.0 >	8	26.5	68	0.6	10	60.9
2.0	33	26.6	72	1.7	8	58.4
3.0	84	25.7	73	1.6	8	59.0
4.0	119	27.4	77	1.8	9	56.6
5.0	131	28.0	77	2.2	9	56.2
6.0	53	26.7	82	2.4	10	56.8
7.0	22	24.7	80	2.2	9	57.0
8.0	13	22.7	78	3.9	7	59.2
9.0	7	24.6	79	2.3	9	57.7
10.0	5	18.2	82	3.1	10	61.9
11.0	2	22.4	86	2.9	10	57.4
12.0	2	22.1	83	5.1	10	60.4

III Season (7-8-9)

λ (10^{-4})	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0 >	1	30.6	68	2.1	8	55.2
2.0 >	68	31.0	64	1.3	5	56.4
3.0	191	31.4	67	1.3	6	55.9
4.0	180	31.0	66	1.5	7	55.3
5.0	149	30.2	66	2.3	7	54.1
6.0	72	29.9	69	2.3	7	53.7
7.0	16	28.9	75	3.0	9	52.2
8.0	7	27.4	82	4.3	10	50.9

IV Season (10-11-12)

$\lambda (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
3.0	5	25.3	79	0.4	10	60.5
4.0	16	22.4	81	2.8	9	63.2
5.0	16	23.7	78	1.7	8	62.9
6.0	34	22.2	81	1.9	9	63.5
7.0	65	21.2	77	2.6	8	63.7
8.0	67	22.0	78	3.2	8	62.8
9.0	45	22.2	79	3.4	7	63.2
10.0	57	22.3	77	3.0	8	61.3
11.0	44	22.2	77	2.7	8	62.8
12.0	34	21.8	76	3.2	7	63.1
13.0	18	21.9	69	3.5	6	63.9
14.0	16	21.2	74	3.4	5	63.7
15.0	7	18.3	79	3.1	10	66.5
16.0	4	17.0	78	2.9	6	67.0

TABLE II.

No. 2 Group of polar conductivity

Negative (λ -) I Season (1-2-3)

$\lambda (10^{-4})$	n	Temp.	Hum.	Wind Vol.	Cloud.	Press.
1.0 >	10	19.7	82	1.1	9	59.9
2.0	67	18.7	73	1.5	8	63.1
3.0	71	17.4	71	2.0	8	65.6
4.0	137	17.4	74	2.9	7	65.6
5.0	100	17.3	73	3.2	7	67.1
6.0	59	15.5	71	3.2	8	66.9
7.0	19	16.5	75	3.3	8	67.0
8.0	7	14.5	80	3.4	9	67.2

Positive (λ_+) I Season (1-2-3)

$\lambda_+ (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0	1	24.4	66	1.0	4	57.0
2.0	12	21.0	74	1.3	8	60.4
3.0	75	18.5	72	1.2	8	63.6
4.0	93	17.3	71	2.1	7	65.7
5.0	125	16.7	75	3.1	7	66.2
6.0	94	15.6	78	3.1	8	65.5
7.0	42	17.5	76	2.5	7	66.0
8.0	20	15.9	75	3.3	9	67.1
9.0	10	16.9	75	3.0	9	67.2

Negative (λ_-) II Season (4-5-6)

$\lambda_- (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0 >	67	26.7	75	1.6	9	58.0
2.0	217	26.8	77	2.0	9	59.2
3.0	158	27.8	77	2.2	9	56.5
4.0	24	27.9	76	2.7	8	58.0
5.0	9	20.5	80	3.8	9	60.7
6.0	2	23.2	89	0.5	10	56.3
7.0	2	26.8	84	2.4	10	57.5

Positive (λ_+) II Season (4-5-6)

$\lambda_+ (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0	38	25.8	70	1.8	8	60.1
2.0	141	26.7	75	1.7	8	57.8
3.0	224	28.1	76	2.1	8	56.5
4.0	49	25.6	83	2.4	9	56.9
5.0	20	22.7	78	3.1	8	59.0
6.0	5	19.6	80	3.8	9	62.4
7.0	2	18.0	86	5.7	10	60.2

Negative (λ_-) III Season (7-8-9)

$\lambda_- (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0	65	31.0	66	1.2	6	56.1
2.0	348	29.8	67	1.3	7	54.1
3.0	231	30.4	66	2.2	7	54.5
4.0	35	28.6	74	3.0	9	51.5
5.0	5	29.4	68	3.2	9	51.9

Positive (λ_+) III Season (7-8-9)

$\lambda_+ (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
1.0	82	30.8	64	1.3	6	51.4
2.0	382	30.9	66	1.5	7	52.3
3.0	195	29.9	69	2.4	7	50.9
4.0	24	29.6	71	3.0	9	52.8
5.0	1	24.8	97	2.3	10	56.4

Negative (λ_-) IV Season (10-11-12)

$\lambda_- (10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
2.0	27	23.7	79	2.0	8	62.8
3.0	67	21.6	79	2.1	9	63.7
4.0	123	22.1	77	3.0	8	63.6
5.0	98	22.6	77	3.0	7	63.2
6.0	76	21.4	79	2.9	8	63.1
7.0	28	21.2	76	3.3	7	66.7
8.0	8	19.5	76	3.3	6	68.5
9.0	1	17.8	68	2.6	3	66.5

Positive (+) IV Season (10-11-12)

$\lambda_+(10^{-4})$	n	Temp.	Hum.	Wind Vel.	Cloud.	Press.
2.0	15	23.5	81	1.9	10	62.8
3.0	45	22.8	82	1.9	9	61.1
4.0	127	21.9	79	2.7	8	63.4
5.0	90	22.1	79	3.2	8	62.8
6.0	90	22.2	75	2.9	7	62.9
7.0	33	21.7	75	3.0	7	62.9
8.0	23	19.7	74	3.1	6	65.2
9.0	4	18.0	68	3.3	3	66.2
10.0	1	15.0	88	1.9	10	68.3

3. Association of Meteorological Groups.

In each season the individual observations have been arranged into groups according to the value of the meteorological elements and having obtained the mean of the conductivity for such a group the association between conductivity and meteorological elements was examined.

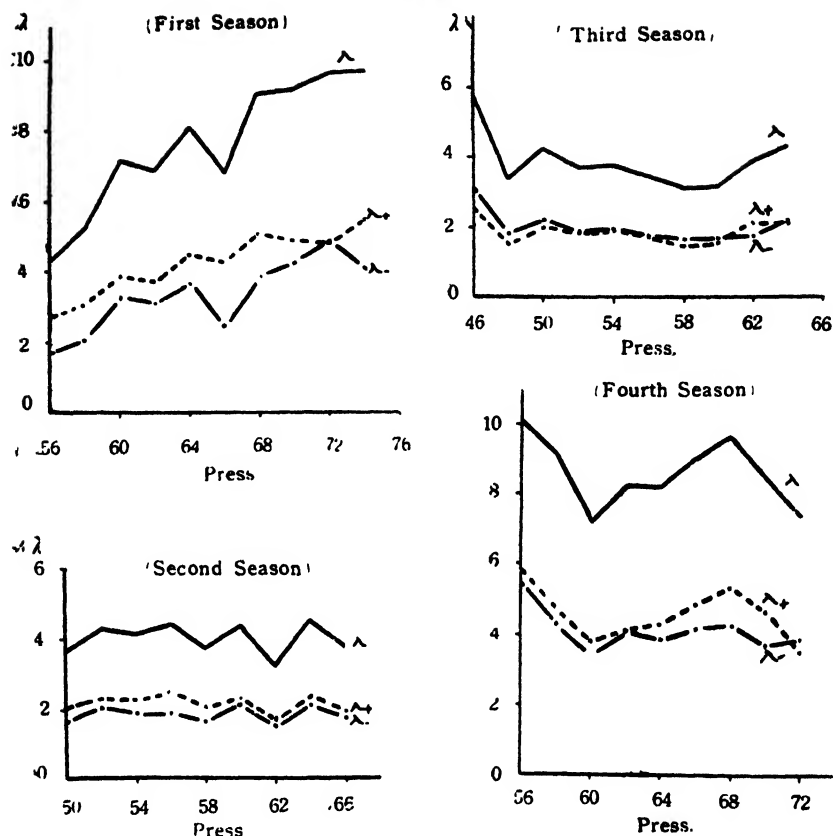
i) *Pressure and conductivity.*

The arrangement has been done into groups of 2 mm. interval, and the results can be seen in tables IIIA and Fig. 3A.

The influence of barometric pressure upon the conductivity is previously, as usual, thought irregular and secondary. "MARKGRAF"⁽³⁾ found from the analysis of the result in Potsdam that the low pressure-area increases conductivity than the high pressure-area, but "HESS"⁽³⁾ could not recognise any such conclusion and showed no relation between conductivity and pressure from the observations in Lans.

Now for our results in Taihoku in the first season the increasing of pressure is associated very regularly with the increasing of conductivity, this shows the characteristic of the winter type. In the

Fig. 3A.

Press.- λ 

second season, such grouped means of conductivity are nearly equal even though pressure is varied and it seems that conductivity as a mean is not so much depended on pressure-groups in this season, owing to the fact that the season is just the interchanging winter type into summer type, and the weather is very unsteady, alternating warm days and cold days simultaneously. In the third season the group of 758 mm. is the minimum of the conductivity, and the larger or smaller groups of pressure than the 758 mm. are almost associated with larger conductivity increased; and for groups of lower pressure the λ_- is larger than the λ_+ , hence q_λ becomes smaller

than unity, showing the characteristic of summer type, being much affected by thunder and typhoonic low.

In the fourth season the maximum conductivity is for 756 mm.-group of pressure and the minimum is for 760 mm.-group. On the group of 770 mm. the λ_+ and λ_- become nearly equal or rather the λ_- somehow larger than λ_+ like as same as in the first season.

As a result such association of pressure-groups is apparently divided into two different types, winter and summer and seems to be much smaller than the influence of wind; and thus our result too is not coincident with MARKGRAF's⁽⁵⁾ conclusion.

ii) *Temperature and Conductivity.*

Concerning temperature with atmospheric electricity "McLAUGHLIN"⁽⁴⁾ showed, denoting absolute temperature "T" and numbers of large ions "N", a relation $NT^{\frac{1}{4}} = \text{constant}$, and HESS⁽⁶⁾ also pointed out some difference in this relation by considering "austasch" effect due to surface temperature of earth; but "ISRAEL"⁽⁶⁾ found that when cold air is blown the number of small ions is greatly increased and the large ions are decreased, but inversely when the air is of a tropical nature large ions become plentiful.

Fig. 3B.

Temp.- λ

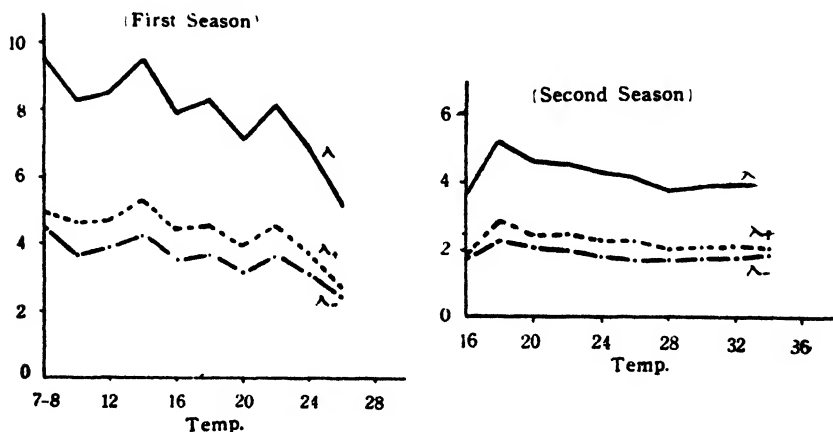
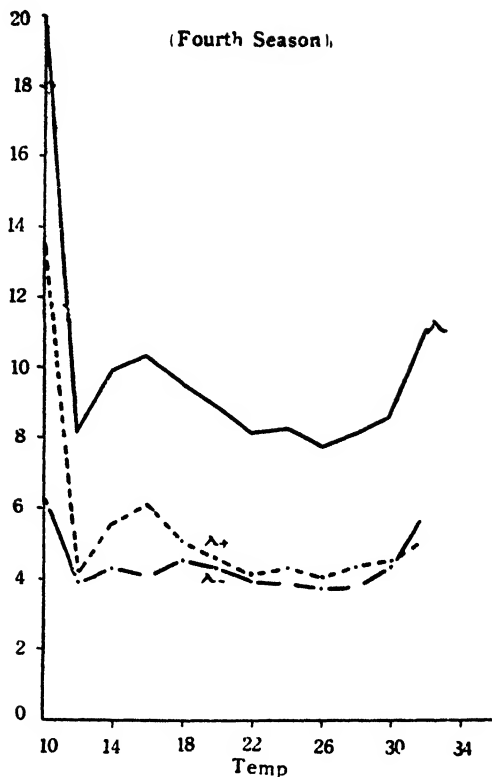
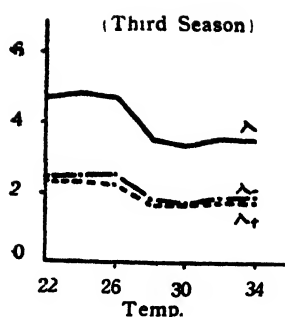


Fig. 3B.

Temp.→



Generally speaking high temperature corresponds to high conductivity. Here we get the mean of conductivity for the group of temperature with interval of 2 degrees as shown in Tables IIIB and Fig. 3B. We can see a considerable regularity between means of conductivity for temperature-groups in each season; as that the group of higher temperature associates with the lower mean of conductivity, but if the temperature-group is above 30 degrees then the conductivity is increased with temperature. For lower temperature below 10 degrees there are no sufficient data to come to any certain conclusion.

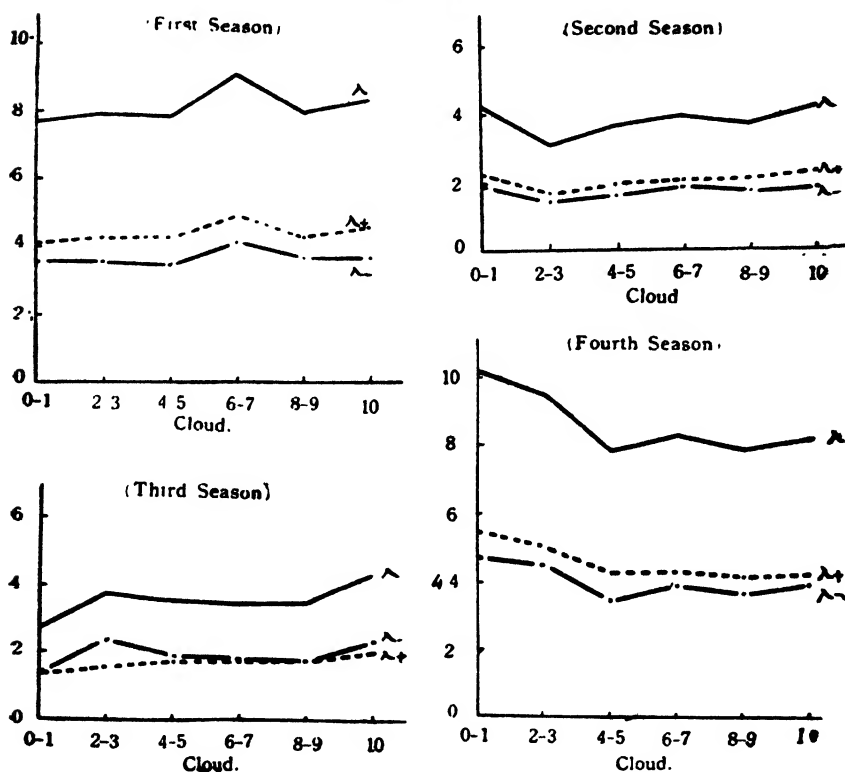
iii) *Cloud Amount and Conductivity.*

Recently,⁽⁶⁾ HESS,⁽⁷⁾ KOSMATH,⁽⁸⁾ MATHIAS⁽⁹⁾ and others discussed the association between conductivity and cloud amount; but this relationship seems to be somewhat indirect, because the variation of cloud amount can cause the variation of other meteorological elements

such as temperature, therefore naturally much regularity cannot be expected in this association.

In our case, groups of cloud amount are selected into intervals of 0-1, 2-3, 4-5, 6-7, 8-9 and 10, for these groups the mean of conductivity are obtained as shown in Table IIIc and Fig. 3C.

Fig. 3C.
Cloudiness- λ



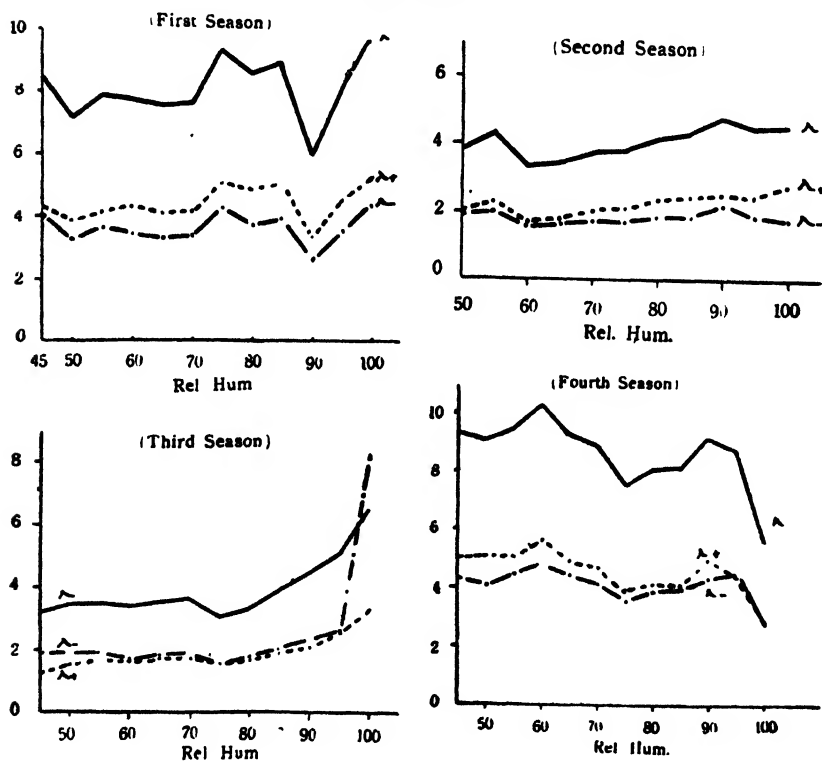
The result is that the conductivity did not give so great a difference due to the variation of cloud amount, and generally the group of cloudiness (10) showed comparatively high conductivity; but in the fourth season in fine weather (a group of cloudiness (0-1)) the conductivity is especially high, and in the third season both polar conductivities become nearly the same as in the group of 4-5, 6-7 and 8-9, therefore the ratio q_λ is almost a unity.

iv) *Relative Humidity and Conductivity.*

The relationship between relative humidity and conductivity is previously discussed under consideration that if relative humidity is⁽⁹⁾ decreased⁽⁹⁾ the small ions are increased, therefore the conductivity become increasing.

By intervals of 5% the relative humidities are grouped in order to examine the association with conductivity as given in the Table IIIb and in Fig. 3D.

Fig. 3D.
Rel. Hum.- λ



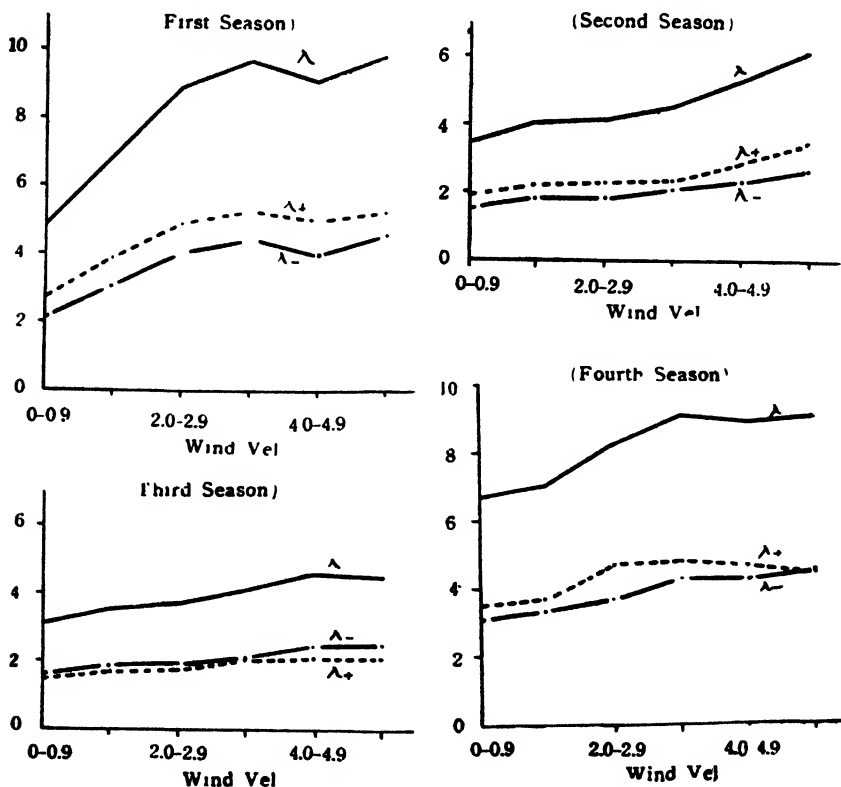
From these results it seems there is a different relation in winter and summer. During the winter season there is no regular association; as that in the first season 90% group of humidity is associated with very small λ , and 75%, 85% or 100% are associated

with relatively large λ , though in the fourth season 75% or 100% is correspondent to a small λ and 60% or 90% is associated with a large λ . But in summer if the relative humidity is increased then the mean of conductivity is almost increased and especially a positive polar conductivity is much more noticeable than the negative. This can be explained from the difference of mobility under the influence of humidity between both ions.

v) Wind velocity and Conductivity.

Wind velocity has been grouped into intervals of 1 m./sec. and the mean conductivity for each group is shown in the Table III. and Fig. 3E.

Fig. 3E.
Wv.-)



The wind velocity was observed at 1 m. height above the ground where the observation of the conductivity was made, so that at a few meters higher than this the velocity should be of course be much greater than this value.

It thus resulted that in general the high conductivity corresponded to the large wind velocity like HESS's⁽³⁾ result. In summer the increasing of conductivity with wind velocity goes nearly linear up to the group of 5 m/sec. and in winter the increasing of λ is very large for lower groups below 4 m/sec. from which velocity it tends to become somewhat smaller as seen in the curve. Such characteristics of winter type may be caused by that the winds above 4 m./sec. are generally accompanied with wet weather of the winter monsoon, and condensation nuclei become so plentiful as to have no effect on λ even though wind velocity is somewhat increased, in spite of the fact that lower wind velocity is much effective by the mixture of air near the earth surface with upper layer and it also excites the respiration of soil so as to quickly make it maximum.

TABLE IIIA
Pressure (700+)

Season	(P-)	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74
I	n							5	17	13	42	98	110	95	64	18	1
	+							1.65	2.14	3.30	3.10	3.65	2.53	3.92	4.27	4.85	4.13
	+							2.70	3.16	3.89	3.78	4.50	4.29	5.11	4.95	4.83	5.57
	+							4.35	5.30	7.17	6.88	8.15	6.82	9.03	9.22	9.68	9.70
II	n				16	25	83	107	95	78	42	11	12				
	+				1.62	2.01	1.92	1.88	1.65	2.06	1.51	2.05	1.70				
	+				2.02	2.34	2.27	2.53	2.08	2.33	1.65	2.38	1.94				
	+				3.64	4.35	4.19	4.41	3.73	4.39	3.16	4.43	3.64				
III	n				70	94	63	181	165	51	11	8					
	+				3.13	1.86	1.92	1.78	1.60	1.61	1.77	2.20					
	+				2.57	1.54	1.85	1.67	1.48	1.49	2.06	2.09					
	+				5.70	3.40	3.77	3.45	3.08	3.10	3.83	4.29					
IV	n							7	35	70	91	83	78	39	18	3	
	+							5.49	4.36	3.40	4.11	3.86	4.21	4.31	3.73	3.90	
	+							5.86	4.80	3.80	4.13	4.34	4.84	5.39	4.69	3.46	
	+							11.35	9.16	7.20	8.24	8.20	9.05	9.70	8.42	7.36	

TABLE III_B
Temperature

Season	(T-λ)	7-8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
I	n	16	42	38	60	70	74	75	64	27	5					
	λ -	4.57	3.64	3.88	4.28	3.50	3.75	3.18	3.63	3.13	2.44					
	λ +	4.99	4.61	4.65	5.25	4.43	4.54	3.97	4.54	3.68	2.69					
	λ	9.56	8.25	8.53	9.53	7.93	8.29	7.15	8.17	6.81	5.13					
II	n					12	27	21	27	41	89	96	90	67	6	
	λ -					1.67	2.34	2.14	2.03	1.87	1.77	1.73	1.78	1.78	1.88	
	λ +					1.83	2.84	2.43	2.45	2.36	2.32	2.02	2.11	2.14	2.08	
	λ					3.50	5.18	4.57	4.48	4.23	4.09	3.75	3.89	3.92	3.96	
III	n								7	17	40	61	199	295	53	
	λ -								2.45	2.49	2.49	1.81	1.74	1.83	1.84	
	λ +								2.32	2.36	2.21	1.69	1.62	1.72	1.67	
	λ								4.77	4.85	4.70	3.50	3.36	3.55	3.51	
IV	n		1	13	10	9	36	81	123	65	37	35	17	1		
	λ -		6.28	3.98	4.30	4.13	4.50	4.28	3.94	3.86	3.66	3.78	4.26	5.96		
	λ +		13.42	4.09	5.58	6.15	5.02	4.59	4.16	4.36	4.01	4.36	4.41	5.09		
	λ		19.70	8.07	9.88	10.28	9.52	8.87	8.10	8.22	7.70	8.14	8.67	11.05		

TABLE IIIc

Cloudiness

Season	(Cloudi- ness—)	0-1	2-3	4-5	6-7	8-9	10
I	n	58	30	19	31	51	273
	λ_-	3.55	3.58	3.47	4.08	3.61	3.70
	λ_+	4.15	4.34	4.34	4.93	4.33	4.62
	λ	7.70	7.92	7.81	9.01	7.94	8.32
II	n	10	15	34	29	64	325
	λ_-	1.97	1.47	1.65	1.87	1.72	1.88
	λ_+	2.27	1.70	1.99	2.02	2.06	2.33
	λ	4.24	3.17	3.64	3.98	3.78	4.21
III	n	51	108	77	82	130	209
	λ_-	1.39	2.19	1.81	1.75	1.72	2.18
	λ_+	1.37	1.58	1.72	1.67	1.70	2.00
	λ	2.76	3.77	3.53	3.42	3.42	4.18
IV	n	34	39	32	29	69	224
	λ_-	4.70	4.56	3.51	3.98	3.74	3.98
	λ_+	5.50	5.03	4.34	4.39	4.22	4.30
	λ	10.20	9.59	7.85	8.37	7.96	8.28

TABLE IIIb
Relative Humidity

Season	(Rel. Hum. -)	45	50	55	60	65	70	75	80	85	90	95	100
I	n	11	25	32	37	48	42	29	36	64	96	59	11
	> -	4.19	3.31	3.68	3.45	3.35	3.41	4.22	3.73	3.91	2.62	3.58	4.39
	> +	4.36	3.88	4.17	4.30	4.13	4.19	5.04	4.84	5.01	3.25	4.51	5.21
	λ	8.55	7.19	7.85	7.77	7.48	7.60	9.26	8.57	8.92	5.87	8.09	9.60
II	n		5	15	27	47	67	53	63	58	64	64	11
	> -		1.90	2.05	1.58	1.61	1.74	1.72	1.82	1.81	2.20	1.93	1.79
	> +		1.99	2.31	1.72	1.80	2.01	2.06	2.33	2.42	2.56	2.50	2.68
	λ		3.89	4.36	3.30	3.41	3.75	3.78	4.15	4.26	4.76	4.43	4.47
III	n	10	27	62	125	128	111	90	44	33	30	19	3
	> -	1.86	1.95	1.89	1.77	1.80	1.83	1.52	1.73	2.02	2.33	2.61	8.20
	> +	1.34	1.59	1.65	1.63	1.73	1.77	1.52	1.60	1.88	2.09	2.49	3.37
	λ	3.20	3.54	3.54	3.40	3.53	3.60	3.04	3.33	3.90	4.42	5.10	6.57
IV	n	2	2	8	19	53	42	59	48	68	55	62	8
	> -	4.36	4.09	4.44	4.71	4.41	4.17	3.53	3.88	3.90	4.26	4.35	2.72
	> +	5.02	5.06	5.02	5.62	4.91	4.69	3.98	4.18	4.16	4.89	4.34	2.76
	λ	9.38	9.15	9.46	10.33	9.32	8.86	7.51	8.05	8.05	9.15	8.09	5.48

TABLE III_E
Wind Velocity

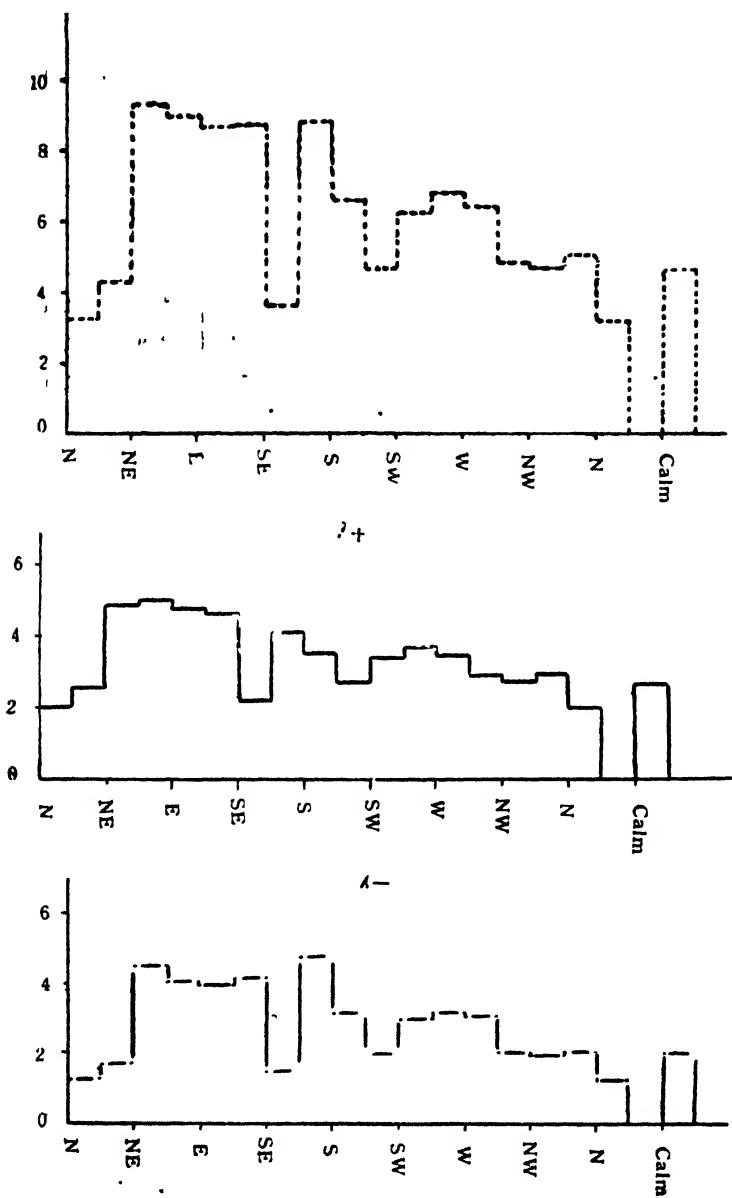
Season	(W. v- λ)	0-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-
I	n	63	87	105	123	62	19
	λ_-	2.16	3.01	4.00	4.45	4.07	4.61
	λ_+	2.76	3.84	4.91	5.28	5.10	5.33
	λ	4.92	6.85	8.91	9.73	9.17	9.94
II	n	144	113	91	72	37	14
	λ_-	1.59	1.85	1.83	2.14	2.35	2.66
	λ_+	1.97	2.20	2.24	2.37	2.90	3.44
	λ	3.56	4.05	4.07	4.51	5.25	6.10
III	n	243	162	150	71	38	21
	λ_-	1.61	1.87	1.91	2.13	2.45	2.44
	λ_+	1.55	1.68	1.78	2.06	2.15	2.13
	λ	3.16	3.55	3.69	4.19	4.60	4.57
IV	n	46	64	96	133	76	13
	λ_-	3.15	3.35	3.61	4.32	4.30	4.59
	λ_+	3.50	3.67	4.70	4.86	4.68	4.54
	λ	6.65	7.02	8.31	9.18	8.98	9.13

4. Wind Direction and Conductivity.

By the influence of the monsoon the predominating direction of wind is extinctly different for winter and summer. In winter the most frequent direction is northeast and in summer, both east and west predominate.

The observed data are grouped into wind direction and for each group the mean values of conductivity and of meteorological elements are taken as in Table IV and Fig. 4.

Fig. 4.
Wind Direction-
I (1-2-3) Season

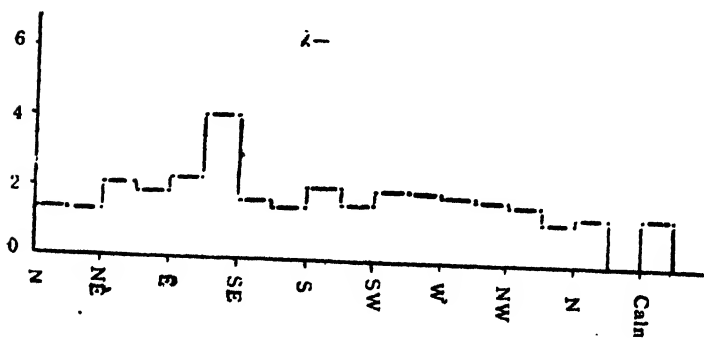
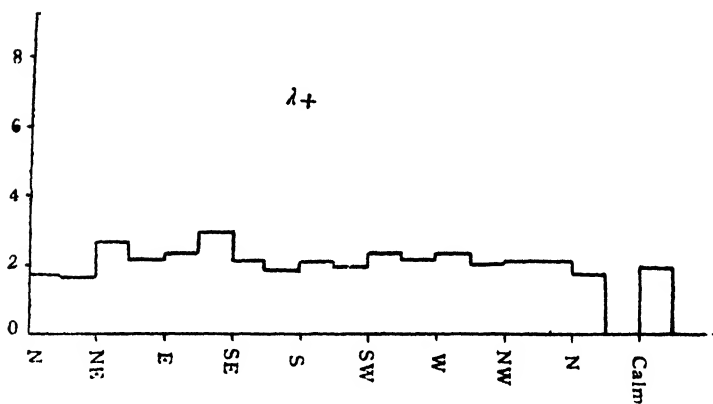


In the first season the conductivity is high for the wind from NE to SSE, the amount reaching up to 9×10^{-4} e.s.u. The lowest is 3.2×10^{-4} e.s.u. of the north wind, and from NWW to NNE the conductivity is comparatively low. The meteorological characteristic for wind direction is that for wind from the NE to ESE, the pressure is relatively high, temperature is rather low and wind velocity is very large, but for wind from the WNW to NNE, the temperature is very high, pressure is rather low and wind velocity is small. Therefore in this season the high conductivity is expected to be associated with high pressure, low temperature and large wind velocity. The positive polar conductivity is higher than the negative in all directions except in that of a SSE wind.

In the second season the highest conductivity is 7.1×10^{-4} e.s.u. of an ESE wind and the lowest is 3.0×10^{-4} e.s.u. of a NNE, one. The wind of higher conductivity is from NE to ESE and the lower is from NNW to NNE. For the wind of high conductivity the mean temperature is low and the wind velocity is large, but on the other hand regarding a wind of low conductivity the mean temperature is rather high and the wind velocity small, so that the one is of the winter type and the other of the summer type; therefore this season is just the interchanging interval of both types and the frequent winds are NE and W. The wind of the winter type shows a comparatively higher conductivity. The positive polar conductivity is slightly higher than the negative in general, but for an ESE wind the negative conductivity is remarkably higher than the positive, which direction is noted to be the same direction of the maximum frequency for thunder showers.⁽¹⁰⁾

The third season is the characteristic season of the summer type and the negative conductivity is rather higher than the positive, especially the negative polar conductivity is much more predominate for the wind of the direction of the high conductivity. The winds from E to S show comparatively high conductivity of 4.5×10^{-4} e.s.u. for these winds the mean temperatures is rather low and the mean wind velocity is large; and the lower conductivity (of order 3.1×10^{-4}

Fig. 4.
II (4-5-6) Season



e.s.u.) is associated with the winds from NNW to NNE for which the temperature is very high and wind velocity is small.

For the fourth season the highest conductivity is 10.7×10^{-4} e.s.u. in a NE wind and the minimum is 4.5×10^{-4} e.s.u. in a NE wind and the minimum is 4.5×10^{-4} e.s.u. in a NNE wind. An easterly wind shows a rather high conductivity and the westerly wind shows a low conductivity. The positive polar conductivity is predominate nearly in all directions of wind except NNE and E. The easterly wind has a large wind velocity, and a NE wind gives a distinguished predominate positive polar conductivity.

On the whole, high conductivity is associated with winds from the NE to SSE for the winter type and with the wind between E and S for the summer type. The characteristic of wind direction for conductivity seems largely to depend on the characteristic purity of air affected by meteorological elements in each direction of wind. Also each wind has a different mean velocity to each other then such a difference in velocity has a strong effect upon the value of the conductivity; but besides this influence it still seems that each wind has a certain characteristic point for the conductivity through all seasons; hence the ionic states are greatly differed by wind direction which may entirely depend on a local nature.

Fig. 4.

III (7-8-9) Season

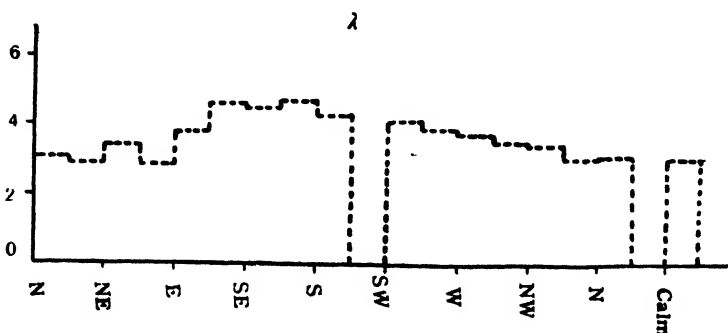
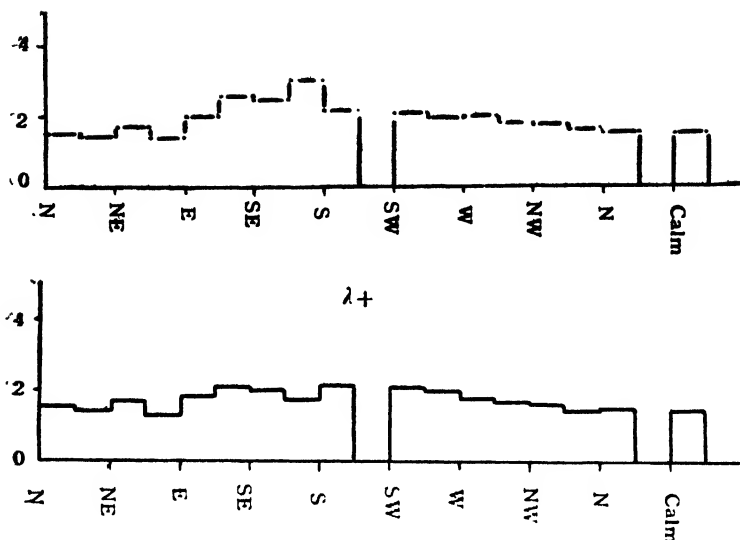


Fig. 4.
III (7-8-9) Season
 $\lambda -$



Previously, HEES⁽⁷⁾ showed the difference of conductivity between seabreeze and landbreeze and the increase of mobility when the wind was NW at Heligoland, and ISRAEL⁽⁶⁾ found the effect of the Föhn wind at München, also KÄHLER,⁽¹¹⁾ NOLAN⁽¹²⁾ and others observed the variation of conductivity related to wind directions; even though these physical meanings and the quantitative relations, are not perfectly solved yet. The purity of air carried by wind differs by wind direction and by different localities such as sea or land and at the same time by the influence of meteorological conditions; then naturally, the life of ions must be differed by wind, so that the conductivity becomes different by wind direction even if the ionising intensity in the air is the same. Moreover, such results should be said to be local.

The difference of conductivity due to wind direction is also affected by wind velocity and seems to almost disappear when the velocity is large enough, for instance in the first season the group of 1-2 meter shows a very large difference of λ according to the

directions but the group of 3-4 m./sec. does not show such a great difference for each direction as seen in the Table IVB.

Here the negative conductivity is comparatively predominant for the wind from ESE to S, though in the air of Paris E. B. DUCLAUX⁽⁹⁾ found that the ratio of both the polar conductivities is maximum for an E wind. The fact that the wind direction of the lower conductivity takes the higher mean temperature may correspond to the result found by ISRAEL⁽⁶⁾ that small ions are increased in cold air and large ions are increased in tropical air.

TABLE IV
Wind Direction—
I (1-2-3) Season

W. Direct.	n	λ_1	λ_2	λ_3	Hum.	Wind Vel.	Cloud	Temp.	Press.
N	3	3.21	1.22	1.99	57	1.0	8	23.5	58.6
NNE	9	4.31	1.73	2.58	67	1.1	3	20.9	62.2
NE	149	9.38	4.51	4.87	77	3.4	1	17.2	65.8
ENE	128	9.02	4.03	4.99	79	3.1	9	14.7	65.4
E	54	8.76	3.99	4.77	62	2.9	7	18.4	65.9
ESE	10	8.79	4.16	4.63	60	4.0	4	18.3	67.6
SE	2	3.63	1.46	2.17	82	1.1	9	14.9	63.8
SSE	5	8.89	4.76	4.13	88	1.2	9	10.0	70.1
S	2	6.67	3.17	3.50	90	0.7	10	12.4	66.4
SSW	3	4.73	1.99	2.74	88	0.9	10	16.6	65.3
SWW	5	6.25	2.87	3.38	78	1.2	8	18.3	64.7
SW	7	6.88	3.17	3.71	74	0.4	8	17.4	64.7
W	16	6.47	3.05	3.42	67	1.8	6	18.9	64.6
WNW	13	4.89	2.00	2.89	69	1.6	7	19.5	63.7
NW	22	4.71	1.96	2.75	66	1.1	8	20.8	59.6
NNW	9	4.94	2.04	2.90	69	1.3	7	20.7	64.6
Calm	31	4.68	2.04	2.64	73	0.0	9	17.2	63.4

Fig. 4.
IV (10-11-12) Season

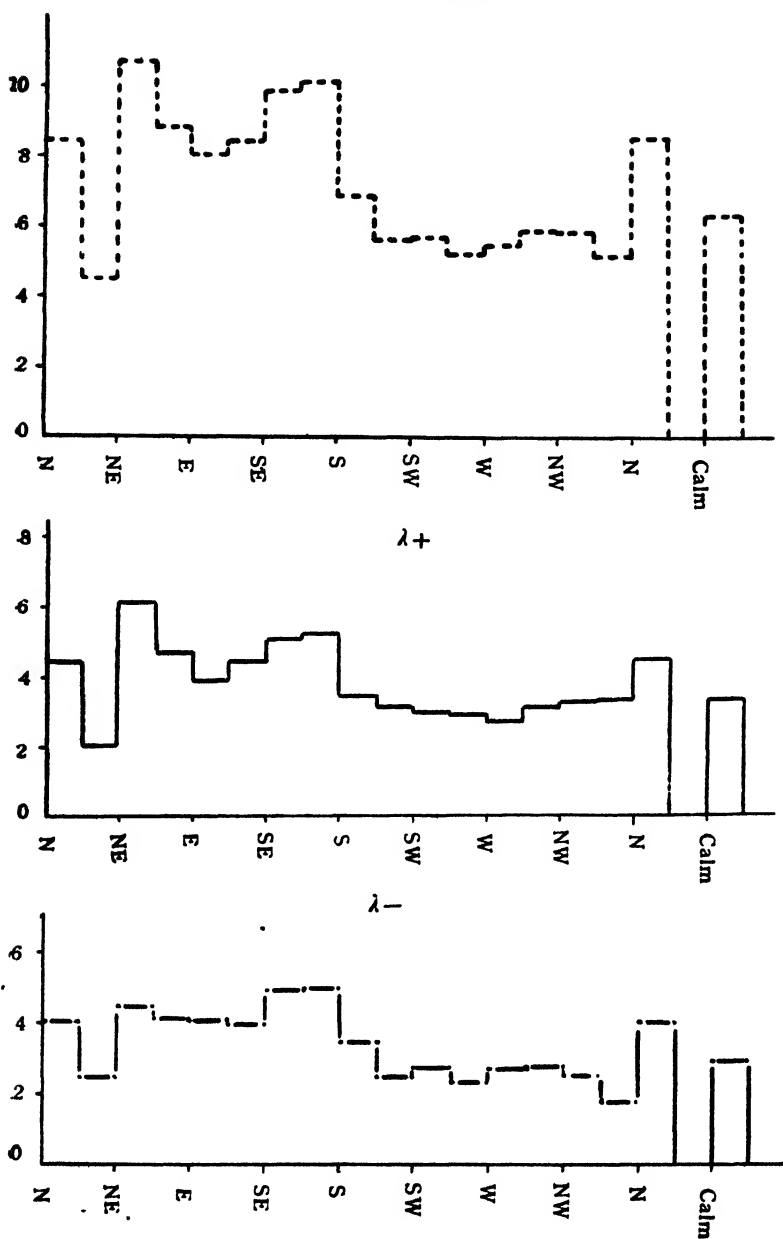


TABLE IV
II (4-5-6) Season

W. Direct.	n	γ	γ_-	γ_+	Hum.	Wind Vel.	Cloud	Temp.	Press.
N	22	3.12	1.41	1.71	73	1.1	8	29.3	57.9
NNE	25	3.01	1.36	1.65	71	1.1	8	28.4	56.4
NE	126	4.83	2.11	2.72	83	3.0	8	24.1	57.6
ENE	31	4.00	1.89	2.11	73	3.6	10	22.3	58.9
E	9	4.65	2.31	2.34	75	2.2	9	24.2	58.4
ESE	3	7.14	4.15	2.99	87	1.5	10	25.0	55.7
SE	6	3.89	1.72	2.17	78	0.8	8	27.7	58.6
SSE	2	3.39	1.55	1.84	78	0.4	10	28.3	58.1
S	1	4.13	2.10	2.03	90	0.7	10	27.4	58.4
SSW	4	3.58	1.60	1.98	92	1.3	10	25.6	56.1
SW	7	4.34	2.03	2.31	79	1.7	9	27.1	56.6
WSW	11	4.16	2.02	2.14	69	2.5	9	29.5	56.0
W	72	4.24	1.93	2.31	70	2.5	9	29.9	55.5
WNW	24	3.80	1.81	1.99	68	2.2	9	29.7	57.1
NW	41	3.73	1.71	2.02	72	1.5	8	28.0	55.9
NNW	10	3.33	1.26	2.07	77	1.5	9	27.9	51.4
Calm	85	3.47	1.51	1.96	81	0.3	9	27.3	55.0

TABLE IV

III (7-8-9) Season

W. Direct.	n	λ	λ_-	λ_+	Hum.	Wind Vel.	Cloud	Temp.	Press.
N	32	3.10	1.56	1.54	66	1.1	7	32.5	58.6
NNE	16	2.89	1.47	1.42	67	1.2	6	31.0	55.9
NE	56	3.44	1.74	1.70	72	2.0	8	29.4	56.4
ENE	45	2.81	1.46	1.35	70	2.4	7	30.1	55.6
E	122	3.83	2.00	1.83	69	2.9	7	29.6	55.3
ESE	37	4.63	2.55	2.08	62	2.4	5	30.7	55.4
SE	21	4.52	2.48	2.04	64	1.9	7	29.2	55.5
SSE	2	4.76	3.00	1.76	54	2.9	9	30.6	53.4
S	4	4.32	2.13	2.19	64	2.4	8	30.0	53.1
SSW	0	0.00	0.00	0.00	00	0.0	0	0.00	00.0
SW	19	4.19	2.02	2.17	53	1.8	5	31.0	53.8
WSW	17	3.95	1.94	2.01	62	1.4	5	31.9	53.2
W	114	3.77	1.97	1.80	65	2.1	7	31.0	53.3
WNW	33	3.53	1.79	1.74	61	1.8	7	31.8	55.2
NW	47	3.48	1.75	1.73	63	1.2	7	31.5	55.1
NNW	6	3.06	1.59	1.47	69	1.6	8	31.3	55.3
Calm	114	3.06	1.55	1.51	68	0.2	7	30.8	56.8

TABLE IV

IV (10-11-12) Season

W. Direct.	n	λ	λ_{-}	λ_{+}	Hum.	Wind Vel.	Cloud	Temp.	Press.
N	7	8.46	4.03	4.43	84	3.2	10	12.6	57.9
NNE	1	4.51	2.48	2.03	64	0.5	10	22.2	67.7
NE	19	10.70	4.53	6.17	84	2.5	9	17.4	66.9
ENE	103	8.84	4.17	4.67	80	3.3	7	22.7	62.3
E	181	8.05	4.15	3.91	78	3.1	8	22.9	62.4
ESE	42	8.45	3.94	4.51	72	2.8	7	23.3	63.2
SE	20	9.92	4.89	5.03	65	2.4	5	26.1	61.4
SSE	6	10.18	4.94	5.24	72	1.5	6	23.4	63.7
S	5	68.9	3.48	3.41	83	0.7	10	22.7	62.6
SSW	1	5.65	2.48	3.17	95	0.2	10	12.6	69.4
SW	5	5.72	2.73	2.99	84	0.9	9	19.2	65.4
WSW	1	5.22	2.30	2.92	69	0.5	5	19.0	67.5
W	7	5.44	2.71	2.73	73	1.4	9	20.1	62.2
WNW	6	5.88	2.76	3.12	69	1.1	8	23.4	62.4
NW	6	5.80	2.54	3.26	77	1.8	6	22.6	64.5
NNW	1	5.09	1.75	3.34	75	1.2	9	24.5	64.1
Calm	10	6.28	2.99	3.29	74	0.3	8	23.6	61.8

TABLE IVB.

I Season
Wind Vel.

Direct.	(1-2)m/sec.		(3-4)m/sec.	
	n	λ	n	λ
N	1	4.12	—	—
NNE	7	4.60	1	8.62
NE	12	7.90	51	10.26
ENE	18	9.05	49	9.48
E	8	8.51	14	8.68
ESE	2	8.26	5	9.49
SE	2	3.62	—	—
SSE	2	9.23	—	—
S	—	—	—	—
SSW	2	5.95	—	—
SW	2	6.24	—	—
WSW	2	8.19	1	8.26
W	6	6.25	3	9.27
WNW	3	5.56	—	—
NW	14	4.85	—	—
NNW	7	5.11	—	—

5. The Ratio of Both Polar Conductivity.

In order to see the association of (q_λ) the ratio of both polar conductivities with meteorological conditions, the observations are arranged into groups of q_λ for which the mean of meteorological elements are taken as seen in the Table V.

The result is that in the first season the group of comparative smaller q_λ associates with larger mean of wind velocity and also of pressure; that in the second season the smaller q_λ corresponds to the larger mean of temperature or relative humidity and also to the

larger mean of wind velocity or pressure, but for very larger q_λ the temperature, humidity is large and wind velocity or pressure is small: and that in the third or fourth season the association between q_λ and meteorological elements is quite irregular.

Therefore it seems there are no significant regular association between the groups of q_λ and the means of meteorological elements; and we only see some tendency that the group of smaller q_λ correspond to the larger means of pressure and wind velocity though the influence of the other factors looks much larger.

The group of maximum frequency is 1.2, for the first season, 1.0 for the second and third season, and 1.1 for the fourth season.

TABLE V

$$q_\lambda = \frac{\lambda_+}{\lambda_-}$$

I (1-2-3) Season

q_λ	n	T	F	Wv	B	P
< 0.6	2	13.2	70	2.3	6	68.4
0.6	1	10.3	88		10	65.3
0.7	1	18.7	81	4.7	10	63.9
0.8	14	17.0	71	3.3	7	65.3
0.9	34	16.8	69	2.3	8	65.7
1.0	53	17.1	73	2.8	8	67.0
1.1	63	17.5	73	2.4	7	66.2
1.2	68	16.9	75	2.9	8	66.3
1.3	58	14.1	75	2.9	8	65.9
1.4	55	16.5	72	2.5	7	65.1
1.5	31	16.4	78	2.3	8	65.4
1.6	32	17.8	76	2.4	8	64.9
1.7	15	15.6	69	3.0	10	66.1
1.8	6	16.9	73	2.2	9	63.5
1.9	7	18.7	74	2.3	8	65.3
>1.9	28	15.7	80	1.8	9	61.3
>2.9	3	19.5	80	1.3	5	62.4
>3.9	1	14.6	96	0.4	10	63.1

TABLE V
II (4-5-6) Season

$q\lambda$	n	T	F	Wv	B	P
<0.6	5	23.0	70	2.6	10	61.1
0.6	3	25.2	72	1.7	9	57.6
0.7	15	27.1	74	2.0	8	57.0
0.8	18	26.7	70	1.9	8	57.5
0.9	30	26.7	78	1.9	8	57.7
1.0	101	26.9	73	1.8	8	58.0
1.1	66	26.8	77	2.3	9	56.8
1.2	42	28.0	73	2.2	9	57.4
1.3	43	27.2	74	2.4	9	57.7
1.4	31	26.2	80	2.2	8	55.8
1.5	32	26.6	78	2.0	9	57.1
1.6	19	27.8	79	2.3	9	56.5
1.7	11	25.6	84	3.0	10	56.2
1.8	16	26.9	82	1.6	10	55.2
1.9	6	26.4	95	1.6	10	55.2
1.9	32	26.7	84	1.7	9	56.2
>2.9	6	26.3	87	1.6	9	55.7
>3.9						
>4.9	2	28.2	80	0.9	8	55.8
>5.9	1	30.4	71	1.3	10	58.5

TABLE V
III (7-8-9) Season

q_A	n	T	F	W_V	B	P
<0.6	32	30.8	62	1.4	6	55.9
0.6	24	31.6	61	1.4	6	55.2
0.7	51	30.0	66	1.9	5	53.8
0.8	76	30.3	67	2.1	7	54.1
0.9	122	30.3	66	2.0	7	54.2
1.0	216	30.9	62	1.6	7	56.1
1.1	51	31.0	66	2.2	6	54.5
1.2	47	30.1	70	1.7	7	55.2
1.3	25	30.4	67	1.6	6	55.7
1.4	14	29.7	71	3.0	8	56.7
1.5	7	28.5	78	0.7	9	52.3
1.6	5	31.4	69	1.6	8	53.2
1.7	6	30.2	74	0.5	8	55.0
1.8	2	32.0	65	1.8	7	54.2
1.9	2	30.8	63	2.6	8	54.5
2.9	3	29.3	67	1.9	5	58.9
>2.9	1	31.3	71	1.1	5	52.5

TABLE V
IV (10 11-12 Season

q_A	n	T	F	W_V	B	P
0.6	2	20.8	85	2.5	5	63.8
0.7	11	20.8	79	2.4	8	64.3
0.8	31	21.3	83	2.6	9	63.2
0.9	47	22.4	83	2.8	8	62.5
1.0	92	22.2	77	2.8	8	63.1
1.1	74	22.6	78	2.9	8	62.5
1.2	58	22.9	75	2.9	8	62.8
1.3	45	20.3	77	2.6	8	64.9
1.4	28	22.1	73	2.5	7	63.6
1.5	16	20.8	73	2.9	8	63.2
1.6	4	20.1	66	2.6	7	67.0
1.7	8	21.4	77	2.3	7	64.3
1.8	5	21.8	74	3.4	7	64.0
1.9	3	18.4	84	2.0	10	66.8
1.9	4	24.6	81	2.0	6	64.9

6. Correlation of Individual Observation.

So far as we have been examining the group-means and have tried to find how the atmospheric electrical conductivity is influenced by the meteorological elements. It may be necessary, however, to show that such results are born out by the individual observations; and thus a statistical analysis of the individual observations serves to show the strength of associations between the conductivity and the meteorological elements by obtaining a measures of correlation.

Considering the daily variation of the conductivity the data are classified into hourly intervals for each season, and the individuals of each interval are treated to see the correlational relation-ship.

Table VIA gives the correlation coefficients between conductivity and meteorological elements, Table VIB the probable errors of them and Table VIC the number of individuals used. These results are concluded in the following.

i) *With wind velocity.*

The total conductivity (λ) and wind velocity are correlated fairly high positive and especially for the first and third season the coefficients are significantly large. Most significant ones among the coefficients are all positive and for 33 in all of them only two are negative, even though the value of these two are very small within their probable errors. The positive polar conductivity (λ_+) has been positively correlated with wind velocity. For the first season the wind towards noon gives a close relation to λ_+ , showing the coefficient larger than 0.6, and for the third season the wind in the afternoon has the most high correlation. The negative polar conductivity (λ_-) is also positively correlated with wind velocity and the coefficients are much remarkable for the first and third seasons, especially the wind in the morning of the third season takes a marked high correlation.

Thus it is concluded that high wind velocity associates with high conductivity.

ii) *With relative humidity.*

The correlation between conductivity and relative humidity is positive and not so strong. However, in summer the coefficients are regular and fairly high and the value are reached from 0.5 to 0.7, in the morning of the third season.

Positive polar conductivity (λ_+) of the summer type takes comparatively regular positive coefficients and particularly they are very strong in the third season. However, in the winter type the coefficients are so small and so irregular that each of them is within the limit of their probable error; hence there are not a able to find any marked relationship.

Negative polar conductivity (λ_-) correlates as similar as the positive polar conductivity; it may be seen that in the winter type the relation is very weak, though in the summer type, especially in the morning of the third season it is far strongly positive. According to the above it appears as a result that the correlation of conductivity with relative humidity is different between summer type and winter type; in winter it is not so significant, but in summer it is well strong positive.

iii) *With temperature.*

The correlational coefficients between conductivity and temperature in Formosa is apparently negative. This point may be different from the usual results. Among these 33 coefficients only 6 are positive but these positive ones are so weak within their probable error. Hence it may be concluded that a lower temperature corresponds to a high conductivity for both of the summer and winter type. It is found the coefficients are negatively very strong in the third season.

Positive polar conductivity shows also negative correlation which coefficient is large in the third season; but negative polar conductivity has much more regular negative coefficients which are very strong. Generally the summer type seems to be correlated negatively

stronger than the winter type, hence it is known that the conductivity is influenced negatively by temperature much stronger in summer than in winter.

iv) *With pressure.*

The relationship between the conductivity and pressure seem to take a different manner for summer and winter; because the correlation coefficients are almost positive in winter and negative in summer, therefore high conductivity is associated with high pressure in winter and inversely with low pressure in summer.

Such results come about by the reason that the direct influence of pressure upon the conductivity is much less than the indirect influence of the secondary effects such as those like the changes of temperature or wind velocity which occur by an accompanying barometric change.

Namely in winter when pressure is becoming high in the monsoon weather the temperature becomes lower and the wind velocity, become greater, therefore a high conductivity may be expected in higher pressure; but in summer when pressure becomes lower the temperature is lower too and the wind velocity is greater owing to typhoons or thunder-storms so that a higher conductivity may be expected in lower pressure. Now the correlation between pressure and temperature is fairly high negative in general, but in the third season of the summer type it becomes well high positive. The correlation between pressure and wind velocity is also usually positive but in the third season of the typical summer type it becomes negative. And also the correlation coefficients between temperature and wind velocity are not so much regular, but they seem to be weakly positive in the first season of the winter type and very strongly negative in the third season of summer type. Elimination of the influences of each other of meteorological factors by partial correlation will be discussed in detail later.

The Table VID shows the correlation coefficients between meteorological factors classified to hourly intervals for each season

and from these coefficients we can see the characteristic relationship between the elements for each season as summarized into the following.

Correlational association between meteorological elements.

	I Season	II Season	III Season	IV Season
Wind velocity)	Positive	Positive (strong)	Negative (strong)	Positive
Pressure)				
Wind velocity)	Positive	Negative	Negative (strong)	Negative (irregular)
Temperature)				
Wind velocity)	Positive	Negative (strong)	Positive	Positive
Humidity)				
Humidity)	Negative	Negative (strong)	Negative (strong)	Negative
Temperature)				
Humidity)	Positive	Positive (irregular very weak)	Negative (strong)	Negative
Pressure)				
Pressure)	Negative (strong)	Negative	Positive	Negative (very strong)
Temperature)				

The numbers of individuals used for correlation are in the Table III

v) *With the ratio of both polar conductivities.*

The correlation between the total conductivity and the ratio (q_λ) of both polar conductivity are not so regular and among these coefficients the larger ones are almost appeared to be negative. The correlations between λ_- and q_λ are very regular and so strongly negative that the high negative polar conductivity corresponds to the small q_λ ; but opposing to this, the correlations between λ_+ and q_λ are not so regular and many of them are positive, particularly larger ones are almost positive.

No certain regular relation between q_λ and the meteorological elements can be found, and nearly half of them are positive and the other half negative; only wind velocity shows a comparatively large negative coefficient; hence a large wind velocity seems to have a tendency of corresponding to small q_λ .

TABLE VIA
 "No. 1" Correlation of γ
 γ_{λ} (Correlation Coeff.)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
γ_{Wv}	I	—	49	65	56	38	51	52	46	78
	II	39	-4	23	21	—	-9	38	30	48
	III	53	67	30	26	84	49	59	42	56
	IV	—	46	24	44	5	32	17	4	17
γ_F	I	—	30	21	4	23	13	9	7	-15
	II	6	3	12	16	—	43	20	4	4
	III	70	51	9	3	36	26	-10	0	33
	IV	—	-6	15	-1	36	10	2	-7	30
γ_T	I	—	8	-17	-8	-13	-43	-32	-23	-28
	II	-27	5	6	-7	—	-24	-14	-11	-43
	III	-77	-64	12	-45	-51	-28	-20	-21	-31
	IV	—	3	8	-14	11	-22	-36	-52	-36
γ_P	I	—	31	24	11	3	51	27	3	33
	II	10	-37	-6	-4	—	-10	-9	7	41
	III	-77	-52	-32	-19	-29	-20	4	-11	-6
	IV	—	-4	-20	17	-33	6	30	39	9

TABLE VIB
 "No. 1" Probable error of γ_{λ}
 Pe_{λ}

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
γ_{Wv}	I	—	93	76	75	145	81	77	80	56
	II	125	102	98	100	—	94	87	91	92
	III	140	79	131	144	53	85	72	92	109
	IV	—	104	116	93	212	102	116	115	143
γ_F	I	—	112	126	108	160	106	105	101	138
	II	146	102	102	102	—	77	97	99	119
	III	99	107	143	155	157	104	110	112	142
	IV	—	131	120	115	186	113	119	114	134
γ_T	I	—	122	128	103	166	88	95	97	130
	II	136	102	103	104	—	89	99	98	97
	III	79	85	142	124	133	103	107	107	144
	IV	—	132	122	113	210	109	104	84	128
γ_P	I	—	111	124	107	169	81	98	102	126
	II	146	88	103	105	—	94	100	99	99
	III	79	105	129	149	165	108	111	111	158
	IV	—	132	118	112	190	114	108	98	146

TABLE VIA
 "No. 2" Correlation of λ_-
 γ_{λ_-} (Correlation Coeff.)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
λ_- Wv)	I	—	42	52	40	35	41	51	39	67
	II	59	4	12	33	—	-11	18	30	56
	III	63	70	28	19	83	47	60	39	39
	IV	—	45	25	30	3	38	29	34	24
λ_- F)	I	—	20	4	-2	29	51	4	11	-21
	II	-19	2	4	2	—	34	14	1	-5
	III	59	57	0	55	32	21	-19	-12	30
	IV	—	-3	24	-1	50	15	9	-1	31
λ_- T)	I	—	-9	-27	-12	-39	-43	-26	-19	-16
	II	-1	10	20	-9	—	-20	-9	-6	-34
	III	-83	-68	22	-51	-44	-26	-27	-18	-35
	IV	—	0	-0	2	0	-24	-46	-45	-30
λ_- P)	I	—	25	33	19	9	59	18	-4	32
	II	23	-36	-25	-16	—	-9	3	8	41
	III	-78	-53	-53	-33	-34	-22	16	-93	8
	IV	—	-4	-24	1	-32	9	39	29	2

TABLE VIB
 "No. 2" Probable Error of γ_{λ_-}
 $P_{e\lambda_-}$

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
λ_- Wv)	I	—	101	96	92	148	90	77	86	78
	II	96	102	102	93	—	94	98	91	82
	III	118	73	133	149	56	87	71	95	135
	IV	—	105	115	105	213	98	109	102	138
λ_- F)	I	—	118	132	108	155	81	105	100	135
	II	142	102	103	105	—	84	99	99	119
	III	127	97	144	108	162	107	107	110	145
	IV	—	132	116	115	160	111	118	115	132
λ_- T)	I	—	122	122	107	143	88	99	98	138
	II	147	101	99	104	—	91	100	99	105
	III	61	77	137	115	145	104	103	108	140
	IV	—	132	123	115	213	107	94	92	134
λ_- P)	I	—	115	118	105	168	70	102	102	127
	II	139	89	96	102	—	94	101	98	99
	III	76	104	104	138	159	107	107	111	158
	IV	—	132	116	115	191	113	101	105	147

TABLE VIA
 "No. 3" Correlation of $\gamma_{\lambda+}$
 $\gamma_{\lambda+}$ (Correlation Coeff.)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
γ_{+} Wv)	I	—	48	65	67	40	60	44	44	77
	II	12	-13	24	17	—	0	32	25	33
	III	48	64	19	30	79	39	52	41	57
	IV	—	40	28	39	-5	35	17	44	8
γ_{+} F)	I	—	36	22	3	14	19	12	7	-3
	II	12	20	19	25	—	39	20	5	8
	III	39	53	24	50	35	19	5	16	38
	IV	—	-7	-2	-7	18	3	-2	-15	10
γ_{+} T)	I	—	-16	-21	6	1	-37	-25	-20	-41
	II	-5	6	-4	-9	—	-22	-8	-10	-45
	III	-73	-65	-2	-43	-51	-25	-17	-16	-23
	IV	—	0	9	-27	-41	-30	-50	-51	-41
γ_{+} P)	I	—	32	17	9	-2	41	23	13	40
	II	-13	-37	-6	-13	—	-11	-17	1	36
	III	-60	-40	-6	1	-11	-24	-10	-18	-14
	IV	—	2	-12	25	-27	11	42	44	13

TABLE VIB
 "No. 3" Probable error of $\gamma_{\lambda+}$
 $P.e.\gamma_{\lambda+}$

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
γ_{+} Wv)	I	—	95	76	60	142	69	85	82	57
	II	145	100	97	102	—	95	91	93	106
	III	150	85	139	141	68	95	81	93	107
	IV	—	109	113	98	212	100	116	93	146
γ_{+} F)	I	—	107	126	103	166	104	104	101	141
	II	145	98	99	98	—	81	97	99	118
	III	185	104	136	116	158	108	111	109	136
	IV	—	131	123	114	203	114	119	112	146
γ_{+} T)	I	—	120	126	103	169	93	99	98	118
	II	146	102	103	104	—	90	100	98	95
	III	91	83	144	126	133	105	103	109	151
	IV	—	131	122	107	177	104	89	85	122
γ_{+} P)	I	—	110	128	103	169	90	100	100	118
	II	145	88	103	103	—	94	98	99	104
	III	125	121	143	155	178	106	110	108	156
	IV	—	132	121	103	197	112	98	93	145

TABLE VIC
No. (n) of Individuals used

Season	Time									
	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h
I	—	30	36	39	16	39	40	44	23	
II	21	44	43	41	—	50	45	46	32	
III	12	22	22	19	14	36	37	36	18	
IV	—	26	30	34	10	35	32	34	21	

TABLE VID
Correlation between meteorological element in each season

γ

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	15h	17h
T-P	I	—	-69	-73	-71	-70	-87	-92	-86	-65
	II	-46	-46	-51	-48	—	-45	-46	-38	-30
	III	63	31	25	-6	20	12	-4	-20	-39
	IV	—	-75	-77	-90	-87	-84	-87	-78	-65
F-P	I	—	-2	1	1	42	20	14	10	-1
	II	11	7	7	16	—	-1	-6	-1	7
	III	-54	-23	-39	-15	-18	-19	-36	-1	25
	IV	—	-23	-9	12	-74	-12	-14	-20	14
Wv-P	I	—	19	18	9	22	6	5	4	43
	II	53	55	73	32	—	26	39	44	45
	III	-89	-59	-26	4	-6	-5	16	-14	-17
	IV	—	20	11	31	14	35	23	29	24
F-T	I	—	-7	-13	-25	-46	-46	-36	-46	-37
	II	-100	-72	-75	-91	—	-58	-52	-12	-91
	III	-68	-92	-100	-82	-71	-86	-90	-82	-81
	IV	—	-26	-59	-43	40	-12	-25	5	-27
Wv-T	I	—	17	-9	20	6	1	17	10	-34
	II	-13	-36	-37	-22	—	11	-18	-21	-12
	III	-60	-72	-35	-45	-37	-23	-7	-36	-5
	IV	—	9	-24	-28	-38	37	-25	-25	-22
F-Wv	I	—	32	17	-7	27	13	-1	5	5
	II	-20	2	2	-17	—	-55	-23	-7	-28
	III	43	57	30	24	18	7	-14	30	-9
	IV	—	26	30	17	15	18	28	19	-27
Wv-F	I	—	32	17	-7	27	-13	-1	5	5
	II	-20	2	2	-17	—	-55	-23	-7	-28
	III	43	57	30	24	18	7	-14	30	-9
	IV	—	26	30	17	15	18	28	19	-27

TABLE VIb
Probable error of γ

$$P.e.r \left(\frac{1}{1000} \right)$$

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
T-P	I	—	64	62	92	86	26	16	27	81
	II	116	80	76	81	—	76	80	85	103
	III	118	130	137	154	173	110	111	108	135
	IV	—	58	50	22	52	34	29	45	85
F-P	I	—	123	132	103	140	104	104	100	141
	II	146	102	102	102	—	95	101	99	118
	III	138	136	122	151	174	108	97	112	149
	IV	—	125	122	113	96	112	117	107	144
Wv-P	I	—	119	128	108	160	108	105	102	115
	II	106	71	48	94	—	89	86	80	95
	III	41	94	134	155	179	112	108	110	154
	IV	—	127	122	104	209	100	113	105	138
F-T	I	—	122	130	102	133	85	92	80	122
	II	0	49	45	18	—	63	74	98	20
	III	105	22	0	51	89	29	21	37	54
	IV	—	123	80	94	179	112	116	115	136
Wv-T	I	—	119	131	105	168	103	103	100	125
	II	145	89	89	100	—	94	98	95	117
	III	125	69	126	124	155	106	110	98	159
	IV	—	131	116	106	182	98	116	108	140
F-Wv	I	—	110	128	103	157	106	106	101	141
	II	141	102	103	102	—	66	96	99	100
	III	159	97	131	146	174	111	109	102	158
	IV	—	123	112	112	203	110	110	109	136
Wv-F	I	—	110	128	103	157	106	106	101	141
	II	141	102	103	102	—	66	96	99	110
	III	159	97	131	146	174	111	109	102	158
	IV	—	123	112	112	203	110	110	109	136

TABLE VI
Correlation of q_λ
 γq_λ (Correlation Coeff.)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
q_λ W_v	I	—	14	-10	-11	-32	8	-36	-7	-4
	II	-62	-38	11	-13	—	15	-3	-14	-45
	III	-9	-50	-19	10	-27	8	-1	-4	15
	IV	—	-6	35	11	2	8	-0	5	-14
q_λ F	I	—	46	26	25	-32	-8	3	-6	43
	II	60	34	24	14	—	-12	27	9	22
	III	-15	-41	29	-8	7	11	33	26	-16
	IV	—	-7	-21	-14	-66	-6	-25	-32	-57
q_λ T	I	—	-25	23	15	37	25	9	11	-16
	II	-23	-3	-26	4	—	17	5	-10	3
	III	-27	48	-35	2	-13	-7	7	0	27
	IV	—	0	6	-29	33	-22	14	-42	-30
q_λ P	I	—	13	44	-19	-32	-46	-5	11	4
	II	-23	-16	15	-15	—	10	-47	-14	-29
	III	21	48	50	60	40	32	-35	-22	-36
	IV	—	3	12	29	-3	13	8	51	20
q_λ λ_-	I	—	4	-25	-32	-38	-42	-28	-32	-37
	II	-21	9	11	16	—	-28	8	-17	-42
	III	-0	-57	-23	-20	-22	14	14	-1	-10
	IV	—	6	23	21	-47	7	5	38	9
q_λ λ_-	I	—	-7	-52	-66	-70	-63	-50	-55	-61
	II	-52	-25	-26	-34	—	-40	-26	-19	-65
	III	-3	-65	-58	-36	-45	-10	-13	-37	-46
	IV	—	-24	-16	-19	-62	-6	3	17	-25
q_λ λ_+	I	—	25	-8	31	-3	-6	-7	-3	-13
	II	3	29	36	39	—	-5	16	27	-18
	III	18	-27	25	4	9	29	51	42	20
	IV	—	30	49	47	-16	49	27	56	42

TABLE VI
 Probable error of $\gamma_{q\lambda}$
 $P.e_{q\lambda}$

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
$\gamma_{q\lambda}$ W_v)	I	—	120	131	107	152	107	92	101	141
	II	90	87	102	103	—	93	101	97	95
	III	193	103	139	153	167	111	111	112	156
	IV	—	131	103	114	213	113	119	115	144
$\gamma_{q\lambda}$ F)	I	—	97	123	102	152	107	105	101	115
	II	94	90	97	103	—	94	94	98	113
	III	191	120	132	154	179	111	99	104	155
	IV	—	131	118	113	120	114	116	103	99
$\gamma_{q\lambda}$ T)	I	—	115	122	107	145	101	105	100	138
	II	139	102	96	105	—	92	101	98	119
	III	181	111	126	155	177	111	110	112	147
	IV	—	132	123	105	190	109	117	95	134
$\gamma_{q\lambda}$ P)	I	—	121	105	105	152	85	105	100	141
	II	136	99	100	102	—	94	79	97	109
	III	186	111	103	99	151	101	97	107	138
	IV	—	132	121	105	213	112	118	85	141
$\gamma_{q\lambda}$ γ_-)	I	—	123	124	97	145	89	98	92	122
	II	141	101	102	102	—	87	100	96	96
	III	195	97	136	149	171	110	109	112	157
	IV	—	131	116	110	166	113	119	98	146
$\gamma_{q\lambda}$ γ_+)	I	—	122	96	61	86	65	80	71	89
	II	109	96	96	93	—	80	94	95	69
	III	196	83	96	135	144	111	109	97	125
	IV	—	124	120	111	131	114	119	112	138
$\gamma_{q\lambda}$ γ_+)	I	—	115	131	99	169	103	105	102	139
	II	147	93	90	89	—	95	98	92	115
	III	189	133	137	155	179	103	82	92	153
	IV	—	120	93	90	207	87	110	79	121

7. Correlation under the Selected Winds.

From the grouped means as above we have already known that in respect to the wind direction the conductivity is apparently quite different from its character, therefore it is necessary to examine the correlation of the individual observations under the condition of the same wind direction. Thus the prevailing wind direction for each season is chosen as like that "NE" wind for the first season and the second season, "E" and "W" wind for the third season and "E" wind for the fourth season; and in each case pressure, temperature, wind velocity, relative humidity and cloud amount are considered for the corresponding meteorological factors to be correlated with the conductivity.

Such correlation-coefficients thus obtained are given in the Table VIIA and their probable errors in the Table VIIB and also the numbers of the individuals in the Table VIIC. From these results the correlational closeness under the selected wind can be summarized as follows:

Correlational Relationship between Conductivity and
Meteorological Elements
Association of the Total Conductivity λ

Wind	Season	Pressure	Temperature	Relative Humidity	Cloudiness	Wind velocity
NE	I	Positive (irregular)	Positive (irregular)	Irregular (more Positive) than Negative	Irregular (forenoon Positive afternoon Negative)	Positive
NE	II	Positive (weak)	Negative (irregular)	Negative	Negative	Positive
W	III	Negative (strong)	Negative	Positive (strong)	Positive	Positive
E	III	Negative	Negative	Negative	Negative	Positive in afternoon weak Negative)
E	IV	Negative	Irregular (weak Positive)	Negative	Negative	Positive

Association of the Negative Polar Conductivity λ_-

Wind	Season	Pressure	Temperature	Relative Humidity	Cloudiness	Wind velocity
NE	I	Irregular (rather Positive)	Irregular (weak Positive)	Irregular (much Positive)	Irregular (some Positive high)	Positive
NE	II	Positive	Negative (irregular)	Negative	Negative	Positive
W	III	Negative (very strong)	Negative	Positive	Positive (strong)	Positive
E	III	Negative (very strong)	Irregular	Negative	Negative	Positive (weak Negative in afternoon)
E	IV	Negative (weak)	Irregular	Negative (irregular)	Negative	Positive

Association of the Positive Polar Conductivity λ_+

Wind	Season	Pressure	Temperature	Relative Humidity	Cloudiness	Wind velocity
NE	I	Irregular (rather negative)	Irregular (rather positive)	Positive	Forenoon Positive afternoon Negative	Positive
NE	II	Irregular (weak positive)	Negative	Negative	Negative	Positive
W	III	Negative	Negative (very strong)	Positive (strong)	Positive	Positive
E	III	Negative	Negative (weak)	Negative (irregular)	Irregular (weak positive)	Positive (afternoon weak negative)
E	IV	Negative (irregular)	Negative	Negative	Negative	Positive

It may be seen that even for the same wind direction the correlation differs not only in value but also in sign sometimes by season in the same wind and also sometimes by wind in the same season. For instance for the NE wind the coefficient of λ with temperature is positive in the first season and negative in the second season; and for the third season the coefficient of λ with relative humidity is strongly positive in the W wind and negative in the E wind.

Such apparent difference in correlation may be due to the influence of the close relationship between each meteorological elements which differ greatly by season or by wind direction, as seen in the Table VIID which is correlation of the meteorological factors of the same individuals used for the above, and in which the elements are related each other like the result as below :

Elements	NE Wind (I Season)	NE Wind (II Season)	W Wind (III Season)	E Wind (III Season)	E Wind (IV Season)
Pressure Humidity)	Positive	Negative	Negative	Negative (irregular)	Negative
Pressure Cloud.)	Positive	Negative	Negative (strong)	Negative	Positive
Pressure Wind vel.)	Negative (weak)	Positive	Negative	Negative	Negative (weak)
Humidity Cloud.)	Positive (strong)	Positive (strong)	Positive (strong)	Positive some negative afternoon)	Positive (forenoon stronger)
Humidity Wind vel.)	Irregular (forenoon and evening pos.)	Irregular (neg. in afternoon)	Positive (strong)	Positive	Positive
Wind vel. Cloud.)	Negative (weak positive in morning)	Negative	Positive	Negative	Negative (irregular)
Pressure Temp.)	Negative (strong)	Negative (strong)	Positive	Negative (positive in morning)	Negative (strong)
Temp. Humidity)	Negative	Negative (strong)	Negative (strong)	Negative (strong)	Negative (positive in evening)
Temp. Cloud.)	Negative	Negative	Negative (strong)	Negative (strong)	Negative (strong)
Temp. Wind vel.)	Positive (irregular)	Negative (irregular in afternoon)	Negative	Negative	Positive

The ratio (q_2) of the both polar conductivities is correlated under the prevailing wind as given in the Table VII E and we may summarize as following :

Correlation of q_λ .

Elements	NE Wind (I Season)	NE Wind (II Season)	W Wind (III Season)	E Wind (III Season)	E Wind (IV Season)
λ	Negative	Irregular	Weak Irregular	Negative	Irregular
λ_-	Negative (strong)	Negative	Negative	Negative	Negative
λ_+	Positive	Positive	Positive	Positive	Positive
Pressure	Irregular (but all high are negative)	Negative	Positive	Positive	Irregular (rather Positive)
Temp.	Irregular	Morning neg. afternoon pos.	Negative	Irregular	Negative in morning or evening
Humidity	Irregular	Irregular	Positive	Positive	Negative
Cloud.	Irregular	Irregular	Negative	Irregular	Irregular (morning weak positive)
Wind vel.	Negative	Negative (morning or evening)	Irregular	Irregular (morning negative)	Irregular (weak negative)

By this result the q_λ for NE wind is seemed to be influenced inversely from wind velocity, and for E wind it is correlated with relative humidity positively in the third season and negatively in the fourth season.

TABLE VIIA

"1" Correlation of λ γ_{λ} (Correlation Coeff.)

Element	Season	Time									
		9h	10h	11h	12h	13h	14h	15h	16h	17h	
λ_P)	I NE	—	33	11	-22	-22	38	1	-5	-49	
	II NE	—	21	21	10	—	3	-0	1	-0	
	III E	-39	-15	-53	0	—	-23	-13	28	—	
	III W	—	-34	-32	—	-76	-53	-11	—	—	
	IV E	—	-15	-24	-10	—	-27	17	20	-30	
λ_T)	I NE	—	9	-10	17	14	-27	-5	31	37	
	II NE	—	-33	-11	-32	—	31	7	1	-35	
	III E	-14	-7	-2	2	—	-4	-4	-39	—	
	III W	—	18	-3	—	-71	-41	-56	—	—	
	IV E	—	7	14	8	—	20	-23	-38	12	
λ_F)	I NE	—	27	33	-14	48	1	-4	-24	-8	
	II NE	—	-28	-7	7	—	-24	-23	-18	9	
	III E	-11	-26	-6	-17	—	-43	1	52	—	
	III W	—	50	22	—	84	44	73	—	—	
	IV E	—	-1	-23	-49	—	-5	-16	-7	14	
λ_B)	I NE	—	50	10	-19	68	-12	-5	-17	-47	
	II NE	—	-35	-42	-14	—	-38	-29	-13	-6	
	III E	8	-6	12	-36	—	-2	-25	20	—	
	III W	—	24	20	—	37	36	55	—	—	
	IV E	—	63	-35	-44	—	-9	-40	-41	-35	
λ_{Wv})	I NE	—	50	47	15	-49	17	12	25	1	
	II NE	—	18	41	27	—	-6	45	36	6	
	III E	54	27	7	60	—	-13	-16	28	—	
	III W	—	27	10	—	83	8	12	—	—	
	IV E	—	16	23	33	—	47	31	32	40	

TABLE VIII

"No. 1" Probable error of γ_A Pe_A (Probable Error)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
P)	I NE	—	160	148	137	178	116	144	254	162
	II NE	—	143	143	149	—	163	174	169	195
	III E	172	159	114	187	—	151	156	180	—
	III W	—	159	135	—	79	97	167	—	—
	IV E	—	135	130	129	—	116	124	125	154
T)	I NE	—	168	149	140	183	125	144	231	184
	II NE	—	134	148	135	—	147	173	169	171
	III E	199	162	159	187	—	159	159	165	—
	III W	—	174	150	—	93	112	116	—	—
	IV E	—	137	135	129	—	120	121	111	167
F)	I NE	—	16	134	141	144	135	144	240	212
	II NE	—	138	149	149	—	154	165	164	193
	III E	200	152	158	182	—	130	159	142	—
	III W	—	135	143	—	55	109	79	—	—
	IV E	—	138	131	99	—	125	125	129	166
B)	I NE	—	127	149	139	101	133	144	248	166
	II NE	—	132	124	147	—	140	159	166	194
	III E	202	162	157	163	—	159	149	187	—
	III W	—	170	144	—	161	118	118	—	—
	IV E	—	83	121	105	—	124	103	108	148
Wv)	I NE	—	127	117	141	142	131	142	239	213
	II NE	—	145	125	139	—	162	139	147	194
	III E	144	151	158	120	—	156	155	180	—
	III W	—	167	149	—	58	134	167	—	—
	IV E	—	134	131	116	—	97	116	117	142

TABLE VIII
 "No. 2" Correlation of λ_-
 γ_{λ_-} (Correlation Coeff.)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
λ_- P)	I NE	—	19	6	-34	-6	51	21	45	-33
	II NE	—	22	33	23	—	10	11	15	26
	III E	-44	-12	-67	-13	—	-31	-29	25	—
	III W	—	-44	-45	—	-65	-60	-14	—	—
	IV E	—	-12	12	-26	—	-31	20	15	-16
λ_- T)	I NE	—	20	-6	3	31	-23	7	-37	37
	II NE	—	-18	2	-32	—	32	0	-1	-44
	III E	-21	3	11	1	—	-9	-4	-37	—
	III W	—	-12	3	—	-54	-53	10	—	—
	IV E	—	6	-14	21	—	7	-24	-29	10
λ_- F)	I NE	—	17	38	-48	73	-7	-12	34	-38
	II NE	—	-25	-11	9	—	-22	-20	23	0
	III E	-7	-37	-6	-39	—	-44	-1	55	—
	III W	—	40	12	—	51	42	51	—	—
	IV E	—	-5	6	-34	—	6	-6	4	16
λ_- B)	I NE	—	40	19	-26	85	-12	-4	3	-51
	II NE	—	-29	-26	-5	—	-23	-23	-10	-9
	III E	11	-16	6	-39	—	-16	-20	23	—
	III W	—	60	20	—	32	39	52	—	—
	IV E	—	-12	-36	-51	—	-0	-41	-43	-37
λ_- Wv)	I NE	—	60	17	8	-75	19	25	47	12
	II NE	—	26	37	52	—	-16	53	37	34
	III E	61	42	5	48	—	-10	-24	23	—
	III W	—	29	10	—	58	14	-1	—	—
	VI E	—	4	-35	40	—	38	33	33	35

TABLE VIIB

'No. 2" Probable error of $\gamma_{\lambda-}$ $Pe_{\lambda-}$ (Probable Error¹)

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
λ_{-P}	I NE	—	163	149	127	186	100	133	203	182
	II NE	—	143	134	142	—	161	172	163	182
	III E	164	161	83	184	—	144	146	183	—
	III W	—	145	120	—	103	85	166	—	—
	IV E	—	136	136	121	—	113	123	127	165
λ_{-T}	I NE	—	162	149	144	169	128	143	220	184
	II NE	—	145	150	135	—	146	174	169	157
	III E	194	163	157	187	—	158	159	168	—
	III W	—	177	150	—	132	97	167	—	—
	VI E	—	137	135	124	—	124	121	119	167
λ_{-F}	I NE	—	164	128	111	87	134	142	225	182
	II NE	—	141	143	149	—	155	167	160	195
	III E	202	141	153	159	—	123	159	133	—
	III W	—	151	148	—	133	111	125	—	—
	IV E	—	133	137	115	—	125	127	130	165
λ_{-B}	I NE	—	142	145	134	52	133	144	255	158
	II NE	—	137	140	149	—	154	165	167	193
	III E	200	159	158	159	—	155	153	185	—
	III W	—	115	144	—	163	114	123	—	—
	IV E	—	136	120	96	—	125	103	106	146
λ_{-Wv}	I NE	—	103	146	143	82	130	135	199	210
	II NE	—	140	129	110	—	159	125	146	172
	III E	127	134	159	144	—	157	150	180	—
	III W	—	165	149	—	124	132	169	—	—
	IV E	—	138	121	103	—	107	114	116	148

TABLE VIIA

"No. 3" Correlation of λ_+ $\gamma_{\lambda+}$ (Correlation Coeff.)

Element	Season	Time									
		9h	10h	11h	12h	13h	14h	15h	16h	17h	
λ_P^+	I NE	—	32	7	-13	-26	3	-21	16	-63	
	II NE	—	18	31	5	—	-4	-7	1	-6	
	III E	-28	-17	-51	17	—	-19	-6	32	—	
	III W	—	-19	-25	—	-55	-44	-7	—	—	
	IV E	—	-15	-14	6	—	-12	15	21	-25	
λ_T^+	I NE	—	7	-7	16	24	-16	5	-7	37	
	II NE	—	-32	-24	-32	—	36	11	-5	-35	
	III E	4	-13	-21	0	—	-2	-5	-38	—	
	III W	—	-22	-8	—	-56	-57	-60	—	—	
	IV E	—	2	6	-70	—	15	-9	-40	4	
λ_F^+	I NE	—	27	49	18	29	14	-22	8	37	
	II NE	—	-26	-1	6	—	-31	-24	-6	6	
	III E	-26	-16	18	8	—	-44	1	57	—	
	III W	—	58	38	—	66	50	80	—	—	
	IV E	—	7	-36	-57	—	-23	-23	-22	7	
λ_B^+	I NE	—	40	18	11	22	-16	-12	-25	-41	
	II NE	—	-34	-2	-19	—	-51	-33	-2	0	
	III E	-2	4	29	-30	—	2	0	6	—	
	III W	—	53	18	—	21	30	48	—	—	
	IV E	—	20	-35	-32	—	-7	-30	-43	-28	
λ_{Wv}^+	I NE	—	32	46	22	-58	7	20	54	-10	
	II NE	—	12	39	31	—	10	37	39	-12	
	III E	45	23	19	68	—	-17	-5	25	—	
	III W	—	17	14	—	70	3	27	—	—	
	IV E	—	9	23	19	—	35	20	25	32	

TABLE VIIB
 "No. 3" Probable error of $\gamma_{\lambda+}$
 $Pe_{\lambda+}$

Element	Season		Time								
			9h	10h	11h	12h	13h	14h	15h	16h	17h
$\lambda_+^P)$	I	NE	—	152	149	142	174	135	138	—	128
	II	NE	—	145	135	149	—	162	173	169	194
	III	E	187	158	118	182	—	153	158	175	—
	III	W	—	174	141	—	131	109	168	—	—
	IV	E	—	135	135	129	—	123	125	124	159
$\lambda_+^T)$	I	NE	—	168	149	140	176	131	144	254	184
	II	NE	—	135	141	135	—	142	172	169	171
	III	E	203	160	152	187	—	159	159	167	—
	III	W	—	171	149	—	128	91	108	—	—
	IV	E	—	133	137	66	—	122	127	109	169
$\lambda_+^F)$	I	NE	—	157	114	139	171	132	137	253	184
	II	NE	—	140	150	149	—	147	164	163	194
	III	E	189	159	154	186	—	128	159	132	—
	III	W	—	120	123	—	105	101	609	—	—
	IV	E	—	137	120	88	—	118	121	124	163
$\lambda_+^B)$	I	NE	—	142	145	142	178	131	142	239	177
	II	NE	—	133	150	145	—	121	155	169	193
	III	E	203	163	146	170	—	159	159	194	—
	III	W	—	129	145	—	179	123	130	—	—
	IV	E	—	132	121	117	—	124	116	106	156
$\lambda_+^{Wv})$	I	NE	—	152	117	137	124	134	138	181	211
	II	NE	—	148	127	136	—	161	150	143	192
	III	E	162	154	153	101	—	154	159	183	—
	III	W	—	175	147	—	95	135	157	—	—
	IV	E	—	137	131	125	—	110	123	122	162

TABLE VIIC
 No. (n) of Mdividuals used

Wind Direction	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
NE {	I	—	16	20	22	13	25	22	19	10
	II	—	20	20	20	—	17	15	16	12
E	III	11	17	18	13	—	18	18	12	—
W	III	—	14	20	13	—	25	16	—	—
E	IV	—	24	24	27	—	29	28	27	16

TABLE VIII

Correlation between Meteorological Element in Each Season

 γ

Element	Season		Time								
			9h	10h	11h	12h	13h	14h	15h	16h	17h
P-B	I	NE	—	68	21	29	22	15	15	10	27
	II	NE	—	-10	10	-12	—	-24	-31	27	-60
	III	E	-36	-44	-14	-21	—	35	-13	3	—
	III	W	—	-95	-54	—	-46	-44	14	—	—
	IV	E	—	56	49	45	—	23	33	21	15
T-B	I	NE	—	-33	-33	-31	-60	-58	-34	-23	-39
	II	NE	—	-29	-29	51	—	-32	-25	26	-18
	III	E	-76	-52	-44	-25	—	-51	-19	-40	—
	III	W	—	-86	-64	—	-52	-51	-68	—	—
	IV	E	—	85	-93	76	—	-61	55	-16	15
F B	I	NE	—	23	62	64	80	62	54	51	41
	II	NE	—	45	68	70	—	49	69	84	80
	III	E	72	51	44	84	—	-57	57	38	—
	III	W	—	81	79	—	69	57	62	—	—
	IV	E	—	88	78	51	—	26	22	57	15
Wv-B	I	NE	—	24	3	-7	-35	-25	14	-26	2
	II	NE	—	-2	-25	34	—	-51	-35	-61	31
	III	E	5	-2	-23	-18	—	-49	-30	24	—
	III	W	—	85	29	—	45	14	14	—	—
	IV	E	—	22	0	-34	—	-50	-61	19	28
T-P	I	NE	—	-40	-54	-79	-82	-74	-100	-73	-83
	II	NE	—	-49	-54	-20	—	-12	-43	-36	-57
	III	E	38	43	13	-14	—	-33	-3	-63	—
	III	W	—	45	39	—	34	20	-26	—	—
	IV	E	—	-72	-70	-73	—	-39	-83	-73	-85

TABLE VIII

Correlation between Meteorological Element in Each Season

Y

Element	Season	Time									
		9h	10h	11h	12h	13h	14h	15h	16h	17h	
F-P	I NE	—	2	45	40	31	-17	-1	29	-22	
	II NE	—	-6	12	-14	—	-40	-29	-23	-5	
	III E	-32	-54	-31	34	—	22	-15	33	—	
	III W	—	-33	-42	—	-31	-27	24	—	—	
	IV E	—	16	-17	-32	—	-45	-38	-19	-20	
Wv-P	I NE	—	-1	17	-1	-6	-44	-45	15	-27	
	II NE	—	62	73	20	—	27	62	57	36	
	III E	-38	-58	-48	-22	—	0	15	-20	—	
	III W	—	-55	-15	—	-35	-24	-61	—	—	
	IV E	—	-33	-35	-22	—	-28	-22	-33	9	
T-Wv	I NE	—	37	-7	26	0	38	-63	14	33	
	II NE	—	-41	-56	-25	—	38	-8	2	-58	
	III E	-17	-18	-16	3	—	1	-1	-40	—	
	III W	—	-65	-38	—	-44	-15	-31	—	—	
	IV E	—	2	5	24	—	45	28	17	9	
F-Wv	I NE	—	31	44	24	-82	6	-14	-4	37	
	II NE	—	20	18	-2	—	-63	-34	-56	65	
	III E	16	12	23	13	—	3	26	15	—	
	III W	—	58	31	—	70	8	25	—	—	
	IV E	—	37	3	3	—	22	16	41	9	
F-T	I NE	—	22	-52	-42	-36	-37	-36	-60	-16	
	II NE	—	-59	-92	-74	—	-67	-66	-59	-82	
	III E	-96	-78	-64	-24	—	-26	-24	-77	—	
	III W	—	-45	-93	—	-50	-100	-91	—	—	
	IV E	—	-45	-23	-8	—	-6	13	11	28	

TABLE VIID

Probable Error of γ

Element	Season		Time								
			9h	10h	11h	12h	13h	14h	15h	16h	17h
P-B	I	NE	—	91	143	132	178	132	141	252	197
	II	NE	—	149	149	148	—	154	157	157	125
	III	E	177	131	156	179	—	140	156	195	—
	III	W	—	176	103	—	147	109	166	—	—
	IV	E	—	95	105	104	—	115	110	124	165
T-B	I	NE	—	145	134	130	120	90	127	235	181
	II	NE	—	137	137	111	—	146	163	158	189
	III	E	86	119	128	175	—	118	153	164	—
	III	W	—	47	89	—	137	100	91	—	—
	IV	E	—	39	11	55	—	79	89	127	185
F-B	I	NE	—	156	92	85	67	83	102	189	177
	II	NE	—	120	81	77	—	124	91	50	70
	III	E	93	121	128	55	—	107	107	167	—
	III	W	—	81	55	—	98	91	104	—	—
	IV	E	—	31	54	95	—	117	122	83	165
Wv-B	I	NE	—	159	150	143	105	127	141	233	213
	II	NE	—	150	141	133	—	121	153	103	176
	III	E	203	163	151	181	—	121	145	183	—
	III	W	—	50	137	—	149	132	166	—	—
	IV	E	—	131	138	115	—	94	80	125	156
T-P	I	NE	—	142	105	54	61	61	0	119	—
	II	NE	—	114	105	144	—	161	142	147	132
	III	E	174	133	156	183	—	142	159	118	—
	III	W	—	144	127	—	165	130	158	—	—
	IV	E	—	67	70	61	—	103	40	61	47

TABLE VIID

Probable Error of γ

Element	Season		Time								
			9h	10h	11h	12h	13h	14h	15h	16h	17h
F-P	I	NE	—	169	120	121	169	131	144	234	203
	II	NE	—	149	148	147	—	137	159	160	195
	III	E	182	115	144	165	—	151	155	174	—
	III	W	—	160	124	—	169	125	159	—	—
	IV	E	—	134	134	117	—	100	110	125	162
W _v P	I	NE	—	169	146	144	185	109	115	249	197
	II	NE	—	92	70	144	—	151	107	114	170
	III	E	174	103	122	173	—	159	156	187	—
	III	W	—	125	147	—	164	127	103	—	—
	IV	E	—	123	121	124	—	115	122	116	168
T-W _v	I	NE	—	146	149	134	187	116	87	250	190
	II	NE	—	125	103	141	—	140	173	169	129
	III	E	197	158	155	187	—	159	159	164	—
	III	W	—	104	128	—	151	132	153	—	—
	IV	E	—	133	133	122	—	100	118	125	163
F-W _v	I	NE	—	153	121	137	61	134	141	254	184
	II	NE	—	144	145	150	—	93	154	116	113
	III	E	193	161	151	181	—	159	148	191	—
	III	W	—	58	135	—	95	131	159	—	—
	IV	E	—	191	133	130	—	119	125	108	163
F-T	I	NE	—	161	110	119	163	117	125	163	207
	II	NE	—	93	23	63	—	90	98	110	64
	III	E	16	64	94	176	—	148	150	79	—
	III	W	—	144	20	—	140	0	29	—	—
	IV	E	—	110	131	129	—	125	125	123	156

TABLE VIII
Correlation of q_λ

Element	Season		Time								
			9h	10h	11h	12h	13h	14h	15h	16h	17h
q_λ)	I	NE	—	23	-61	-20	-73	-40	-36	13	-33
	II	NE	—	29	44	-6	—	-15	-10	27	22
	III	E	-51	-5	-49	-6	—	6	-4	29	—
	III	W	—	19	-17	—	18	11	-0	—	—
	IV	E	—	22	-19	-19	—	33	-24	-83	13
q_λ)	I	NE	—	-19	-82	-86	-23	-60	-63	-70	-62
	II	NE	—	12	-8	-27	—	-43	-40	-7	-15
	III	E	-61	-29	-48	-45	—	-8	-34	18	—
	III	W	—	-26	-33	—	6	-29	-39	—	—
	IV	E	—	-13	-46	-83	—	2	-57	-30	-37
q_λ)	I	NE	—	47	-39	52	67	14	-42	-6	10
	II	NE	—	47	63	6	—	13	4	37	50
	III	E	-27	21	-20	14	—	23	30	49	—
	III	W	—	59	17	—	34	47	40	—	—
	IV	E	—	49	24	15	—	65	-5	21	48
q_λ P)	I	NE	—	19	9	22	-34	-56	22	-51	6
	II	NE	—	2	32	-54	—	-4	-35	-42	-24
	III	E	45	-13	39	41	—	13	31	-1	—
	III	W	—	34	30	—	9	25	7	—	—
	IV	E	—	-10	35	46	—	14	-27	5	-11
q_λ T)	I	NE	—	-19	18	1	54	8	-79	39	-19
	II	NE	—	-29	-39	31	—	7	37	4	3
	III	E	70	-31	-54	3	—	22	-1	12	—
	III	W	—	-4	-22	—	-10	-9	-26	—	—
	IV	E	—	-2	-27	-29	—	29	43	-12	-10
q_λ F)	I	NE	—	17	-19	45	-28	27	12	-46	63
	II	NE	—	-9	12	-21	—	-8	-18	19	-6
	III	E	-40	41	46	52	—	-5	12	20	—
	III	W	—	12	29	—	43	8	34	—	—
	IV	E	—	33	-35	-30	—	-46	-8	-46	-11
q_λ B)	I	NE	—	-0	8	25	-21	8	-2	-32	25
	II	NE	—	-9	20	-42	—	-27	-11	13	5
	III	E	-43	39	22	-2	—	28	31	-45	—
	III	W	—	-11	-3	—	-25	-27	-13	—	—
	IV	E	—	46	18	24	—	-18	13	-6	9
q_λ W)	I	NE	—	-17	-1	4	-2	-4	-45	-26	-43
	II	NE	—	-20	10	0	—	32	-11	-4	-70
	III	E	-40	-45	29	31	—	-39	28	-17	—
	III	W	—	-16	-3	—	32	-5	37	—	—
	IV	E	—	2	6	-36	—	11	-14	-16	-8

TABLE VIII
Probable Error of q_λ

Element	Season	Time								
		9h	10h	11h	12h	13h	14h	15h	16h	17h
q_λ)	I NE	—	160	94	138	87	113	125	251	190
	II NE	—	137	121	149	—	159	172	157	186
	III E	150	163	121	186	—	158	159	179	—
	III W	—	174	146	—	181	133	169	—	—
	IV E	—	131	133	125	—	111	121	40	166
q_λ)	I NE	—	163	49	37	177	86	87	130	131
	II NE	—	148	149	139	—	133	146	168	191
	III E	127	149	122	149	—	156	141	189	—
	III W	—	168	128	—	186	124	143	—	—
	IV E	—	136	109	40	—	125	86	118	146
q_λ)	I NE	—	132	127	105	103	132	119	254	211
	II NE	—	117	81	149	—	160	174	146	146
	III E	188	156	153	183	—	151	145	148	—
	III W	—	117	146	—	165	105	142	—	—
	IV E	—	105	130	127	—	72	128	124	130
q_λ P)	I NE	—	163	149	137	165	93	137	189	212
	II NE	—	150	135	106	—	162	153	139	184
	III E	161	160	135	156	—	156	144	195	—
	III W	—	159	137	—	186	127	168	—	—
	IV E	—	137	121	102	—	123	119	130	167
q_λ T)	I NE	—	163	145	144	132	134	—	216	205
	II NE	—	137	127	136	—	162	150	169	195
	III E	104	147	113	187	—	151	159	192	—
	III W	—	180	143	—	185	134	158	—	—
	IV E	—	138	128	119	—	115	104	128	167
q_λ F)	I NE	—	164	145	115	172	125	142	201	128
	II NE	—	149	148	143	—	162	168	163	194
	III E	171	136	125	137	—	159	157	187	—
	III W	—	177	137	—	152	134	149	—	—
	IV E	—	123	120	118	—	99	127	102	167
q_λ B)	I NE	—	169	149	135	179	134	144	229	200
	II NE	—	149	144	124	—	151	172	166	195
	III E	165	138	151	187	—	147	144	156	—
	III W	—	178	150	—	175	125	166	—	—
	IV E	—	109	134	122	—	121	126	129	168
q_λ Wv)	I NE	—	164	150	144	187	135	165	238	174
	II NE	—	144	149	150	—	146	172	169	99
	III E	171	130	146	169	—	135	147	189	—
	III W	—	175	150	—	168	135	146	—	—
	IV E	—	138	137	113	—	124	125	127	168

8. Partial Correlation of the Conductivity

The fact from the above two sections having considered the influence of daily oscillation and a difference of wind direction in which the correlational coefficient is much different by a season not only in magnitude but in sign must be naturally introduced that the association between the conductivity and the meteorological factors should be a resultant influence of the each factor with others, hence it makes apparently a large difference for their correlation and that the one among all meteorological factors which plays a principal influence to the conductivity might be differed by season. Therefore we must try to find which element is a main factor to make a close relation with the conductivity after eliminating the influences of the other factors by the method of partial correlation. For this purpose we have treated the correlations of individual data at 10h and 14h in the diurnal variation of the first season as a winter type and those of the third season as a summer type, and the three meteorological elements pressure, temperature and wind velocity are considered to take the partial correlation of the total conductivity.

Let the notations of pressure and temperature and wind velocity be p. t. w, respectively and the correlation coefficient is denoted like that $\gamma_{\lambda p \ t w}$ is the correlation coefficient between conductivity and pressure after having eliminated the influences of temperature and wind velocity, and such a coefficient is calculated from the equation like as the first order

$$\gamma_{\lambda p \ t} = \frac{\gamma_{\lambda p} - \gamma_{\lambda t} \gamma_{pt}}{(1 - \gamma_{\lambda t}^2)^{1/2} (1 - \gamma_{pt}^2)^{1/2}}$$

and also the second order

$$\gamma_{\lambda p \ t w} = \frac{\gamma_{\lambda p \ t} - \gamma_{\lambda w \ t} \gamma_{pw \ t}}{(1 - \gamma_{\lambda t \ w}^2)^{1/2} (1 - \gamma_{pw \ t}^2)^{1/2}}$$

Now from the Table VI we can pick up the following results.

Apparent Correlation Coefficients.

	Winter (1st season)		Summer (3rd season)	
	10h	14h	10h	14h
$\gamma_{\lambda t}$	-0.08	-0.43	-0.64	-0.28
$\gamma_{\lambda p}$	0.31	0.51	-0.52	-0.20
$\gamma_{\lambda w}$	0.49	0.51	0.67	0.49
γ_{tp}	-0.69	-0.87	0.31	0.12
γ_{tu}	0.17	0.01	-0.72	-0.23
γ_{pw}	0.19	0.06	-0.59	-0.05

From these coefficient, the influence of one element is drawn out, and then the coefficients have become as follows.

Partial Correlation of the First Order.

	Winter (1st season)		Summer 3rd season	
	10h	14h	10h	14h
$\gamma_{\lambda t p}$	0.195	0.032	-0.590	-0.263
$\gamma_{\lambda p t}$	0.353	0.305	-0.440	-0.175
$\gamma_{\lambda t u}$	-0.190	-0.494	-0.306	-0.197
$\gamma_{\lambda u t}$	0.510	0.570	0.392	0.456
$\gamma_{\lambda p, u}$	0.265	0.558	-0.208	-0.179
$\gamma_{\lambda u p}$	0.462	0.558	0.527	0.551

It is noted that the correlation coefficient between conductivity with temperature eliminating the influence of wind velocity is negative in summer or in winter, and the coefficients with wind velocity excluding out the effect of temperature or pressure are positive; but other coefficients $\gamma_{\lambda t p}$, $\gamma_{\lambda p t}$ and $\gamma_{\lambda p, w}$ are seemed apparently to have opposite sign for summer type and for winter type.

Now then the correlational relation of the conductivity with the meteorological conditions which is eliminated the influences of two factors among the three meteorological elements has been tried and the result is found as below:

Partial Correlation of the Second Order.

	Winter (1st season)		Summer (3rd season)	
	10h	14h	10h	14h
$\gamma_{\lambda p, t, u}$	0.012	-0.018	-0.385	-0.182

$\gamma_{\lambda p, tw}$	0.189	0.309	-0.291	-0.162
$\gamma_{\lambda w, tp}$	0.426	0.558	0.202	0.523

Thus it can be known that the principal elements which gives main influences to the conductivity is wind velocity in both summer type and winter type and takes a high positive correlational coefficient, so that the larger wind velocity is associated with high conductivity.

In winter type the correlation with pressure is the second large which is positive, and the coefficient with temperature is so small that it is not worthy to consider of its influence. The coefficients at 14h are little larger than those at 10h.

But in summer type the coefficients with temperature and with pressure are both negative. The influence of temperature on conductivity is far larger than that in winter; especially in morning (at 10h) the coefficient of temperature is maximum and larger than the coefficient of wind velocity and becomes the principal factor; though in afternoon the coefficient of wind velocity is much bigger than that of temperature and which is still seemed as the main influence upon the conductivity.

The positive high closeness of wind velocity may come from that the increase of wind velocity excites the respiration of soil to bring out the ionized air from earth and also make the air clean by mixing of upper air with lower air, so that the result is to increase conductivity; and the negative closeness of temperature in summer may be that the rise of temperature, being accompanied by the strong solar radiation and the difference of temperature between soil surface and air being much larger, excites the thermal convection of air on soil, and make easier to bring dust into upper layer, and therefore the conductivity becomes low, though in afternoon this temperature-association is not so remarked because summer rain is very frequent only in afternoon and wet the earth.

9. Effect of Rain

By the observation at Glencree near Dublin the excess of negative

ions during rain, especially its abnormal increase in a very heavy rain was noted by Nolan.⁽¹⁸⁾ But Hess and Kosmath⁽⁹⁾ showed that the influence of rain is very significant, the conductivity is decreased by rain and becomes minimum if the ground is sufficiently wet after rain, and that the λ_- is decreased more than the λ_+ , therefore the ratio q_λ becomes larger, because the negative ion is absorbed by misty rain stronger than the positive.

In our case the result of observations here led us to the following different conclusions. The average value of the conductivity during rain and also those averaged for about 5 hours before or after rain are given in the Table IX.

During rain the conductivity of atmosphere is very fluctuable, the λ_- is more changeable than the λ_+ and the influence is seemed to depend on the intensity and duration of rain.

An inspection of the Table IX will clearly show that in a case of a long continued drizzly rain like a monsoon rain the conductivity as a average during rain is decreased and becomes much smaller after rain if the earth is sufficiently wet; but in a case of heavy rain like that during thunder storm the increase of the conductivity is observed and the rate of increase is larger according to a greater intensity and shorter duration of rain.

After heavy rain the conductivity becomes generally smaller than that before rain; though, sometimes, in exceptional case it seldom increases after thunderstorms.

In a drizzly rain small ions, especially negative ones collide with condensational nuclei, therefore the decrease of λ_- is significant; but contrarily in a heavy rain the conductivity is increased by increase of ions produced by Lenard effect, splashing of the rain-drops, hence the increase of the λ_- is much more significant.

If the earth is perfectly wet after rain and the respiration of soil is reduced the content of emanation from ground becomes to be much less, so that the decreasing the conductivity should be caused.

If it is misty drizzle the Aitken nuclei (N) may be possible to be increased, then if the rate of production of ions is remained con-

stant the number of small ions must be decreased ; but if rain is heavy shower the dust-neuclei may be decreased, therefore the number of small ions (n) must be increased as known from the equilibrium equation even if the production of ions is assumed constant, though practically there occurs another source of the ionic production so-called as Lenard effect.

TABLE IX
1931

No.	Date		\bar{p}	$\bar{p} -$	$\bar{p} +$	q_A	Precipitation	Duration	Mean Intensity	N. B.
No. 1	June 26	Before	2.65	1.33	1.33	1.00				
		During	6.37	3.49	2.88	0.83	102.8	6.40	15.4	Thunderstorm
		After	3.48	1.65	1.83	1.11				
No. 2	July 2	Before	3.89	1.99	1.90	0.96	71.4	8.25	8.6	
		During	4.05	2.05	1.99	0.97				Shower
		After	3.61	1.68	1.93	1.15				
No. 3	July 10	Before	2.84	1.47	1.37	0.93	81.6	7.10	11.4	Thunderstorm
		During	4.25	2.18	2.07	0.95				
		After	1.79	0.88	0.91	1.03				
No. 4	Aug. 14-15	Before	5.28	2.74	2.53	0.92	4.2	9.40	0.5	
		After	5.45	3.02	2.43	0.81				
		Before	3.83	2.16	1.67	0.77	22.0	60.30	0.4	
No. 5	Aug. 17-20	During	2.95	1.44	1.51	1.05				Long continued light rain
		After	2.50	1.33	1.17	0.88				
		Before	3.22	1.67	1.55	0.93	180.7	55.00	3.3	Typhoon
		During	5.70	2.98	2.72	0.91				
		After	2.72	1.32	1.40	1.06				

TABLE IX
1931

Date	γ	$\gamma -$	$\gamma +$	q_λ	Precipitation	Duration	Mean Intensity	N. B.
No. 7 Sept. 22-23	Before	3.97	2.01	1.97	0.98	91.8	37.30	Typhoon
	During	5.53	3.09	2.44	0.79			
	After	2.77	1.19	1.58	1.33			
No. 8 Oct. 19-22	Before	9.62	4.85	4.77	0.98	88.2	82.00	Monsoon rain
	During	8.92	4.53	4.39	0.97			
	After	6.30	2.91	3.39	1.17			
No. 9 Dec. 3-4	Before	8.46	3.87	4.58	1.18	19.0	24.00	0.8
	During	9.80	4.75	5.05	1.06			
	After	8.24	4.27	3.97	0.93			

TABLE IX
1932

Date	γ	$\gamma -$	$\gamma +$	q_λ	Precipitation	Duration	Mean Intensity	N. B.
No. 1 Jan. 12-13	Before	9.20	4.43	4.78	1.08	5.8	10.40	Shower
	After	9.47	4.52	4.95	1.10			
No. 2 Feb 9	Before					29.9	19.20	1.5

TABLE IX
1932

Date		γ	$\gamma -$	$\gamma +$	q_A	Precipitation	Duration	Mean Intensity	N. B.
No. 3 Feb. 17-19	During	9.84	4.56	5.28	1.16				
	After	7.86	3.68	4.19	1.14				
	Before	10.26	4.67	5.59	1.20	15.6	49.00	0.3	Monsoon rain
	During	8.78	3.91	4.87	1.25				
	After	7.99	3.95	4.04	1.02				
No. 6 March 30-31	Before	4.00	1.64	2.35	1.43	23.3	17.50	1.3	Thunder storm
	During	6.08	3.25	2.83	0.87				
	After	6.77	2.79	4.18	1.50				
	Before	6.78	3.00	3.79	1.26	7.7	33.25	2.5	Shower
	After	5.61	2.37	3.24	1.37				
No. 7 April 5	Before	9.59	4.36	5.23	1.20	28.6	14.05	2.0	Thunder storm
	During	9.91	4.21	5.70	1.35				
	After	9.63	4.06	5.57	1.37				
	Before	3.34	1.73	1.61	0.93	105.4	30.40	3.0	
	During	3.68	1.78	1.90	1.07				
No. 8 April 6	After	2.32	1.11	1.21	1.09				
	Before	3.28	1.26	2.02	1.61	4.7	4.50	1.0	Shower
	During								
	After								
	Before								
No. 9 April 12-14	During								
	After								
	Before								
	During								
	After								
No. 10 May 12	Before								
	During								
	After								
	Before								
	During								

TABLE IX
1932

Date		γ	$\gamma -$	$\gamma +$	q_A	Precipitation	Duration	Mean Intensity	N. B.
No. 11 May 24-26	During	4.03	1.91	2.12	1.11				
	After	3.20	1.19	2.01	1.69				
	Before	4.64	2.17	2.46	1.13	21.8	40.40	0.5	Long continued rain
	During	3.07	1.27	1.80	1.42				
	After	3.00	1.39	1.60	1.15				
No. 12 June 6-9	Before	4.87	1.96	3.01	1.62	165.5	64.40	2.6	
	During	5.47	2.18	3.29	1.51				
	After	3.11	1.10	2.02	1.81				
	Before	3.56	1.36	2.20	1.62	30.6	8.40	3.5	Thunder storm
	During	4.98	2.42	2.56	1.06				
No. 13 June 14	After	3.47	1.35	2.12	1.57				
	Before	4.11	1.96	2.15	1.10	11.8	2.00	5.9	Thunder storm
	During	4.24	2.08	2.16	1.04				
	After	3.91	1.74	2.17	1.25				
	Before	3.98	1.96	2.02	1.03	122.1	6.15	19.5	Thunder storm
No. 14 June 28	During	5.87	2.80	3.07	1.10				
	After	3.00	1.45	1.55	1.07				
	Before								
	During								
	After								
No. 15 June 5	Before								
	During								
	After								
	Before								
	During								

10. Variation during Thunderstorm

Having found that the conductivity of air is extremely high in the morning of the thunder-days A. GÖCKEL⁽¹⁷⁾ and SCHWEIDLER suggested to use this phenomena for forecasting of local lightnings, but in North Germany this evidence was shown not to be applied by BUDIG⁽¹⁴⁾ or MARKGRAF,⁽⁸⁾ and also recently HESS⁽¹⁾ and KOSMATH did not agree with this from the results at Lans by the reason that even if the λ is very high in the morning it becomes sometimes föhn, sometimes thunder and that even though it became thunderstormy in the afternoon the λ in the morning of the day was often very low.

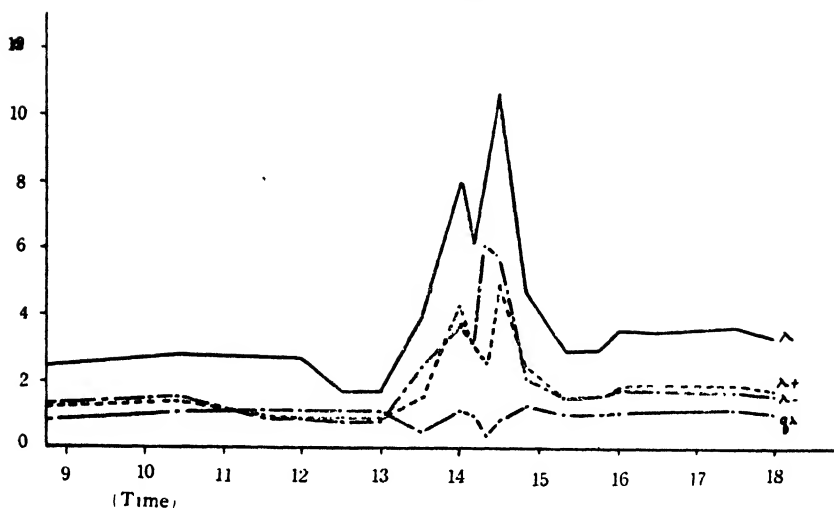
During from June 1931 till July 1932 we got the results of observations of the conductivity for 41 days of afternoon-thunderstorm. There were no thunders between October and February. Thunderstorms⁽¹⁰⁾ were most frequent in July and March. Having examined these 41 thunder-days whether the conductivity of air is specially high in the morning or not, we got the result of the Table X_A where the conductivities (λ , $\lambda -$, and $\lambda +$) are the means in the interval from $8\frac{1}{2}^h$ to $10\frac{1}{2}^h$ of each day, q_λ is the ratio of both polar conductivities, R is the ratio of the total conductivity in the morning before the thunder to each monthly mean of the same time-interval, and the last column is the time of beginning of the thunderstorm.

As seen clearly in the Table it cannot be sure that the conductivity in the morning of the thunder-day is abnormally high; but among these 41 results, 6 are nearly normal, 13 especially high, and the other 22 very low. For example on July 9th, 1931 the conductivity was normal in the morning though the thunderstorm was occurred at $14^h 45^m$; and on April 6th 1932 it was extremely high and on March 11th abnormally low.

During thunderstorm we often observed abnormal large variations of the conductivity. Here we show two thunders as typical examples on June 26 and August 14, 1931 for which the data are in the Table X_B.

Fig. 10_B No. 1 is the graphical representation of the variation of

Fig. 10_B
No. 1. June 26.

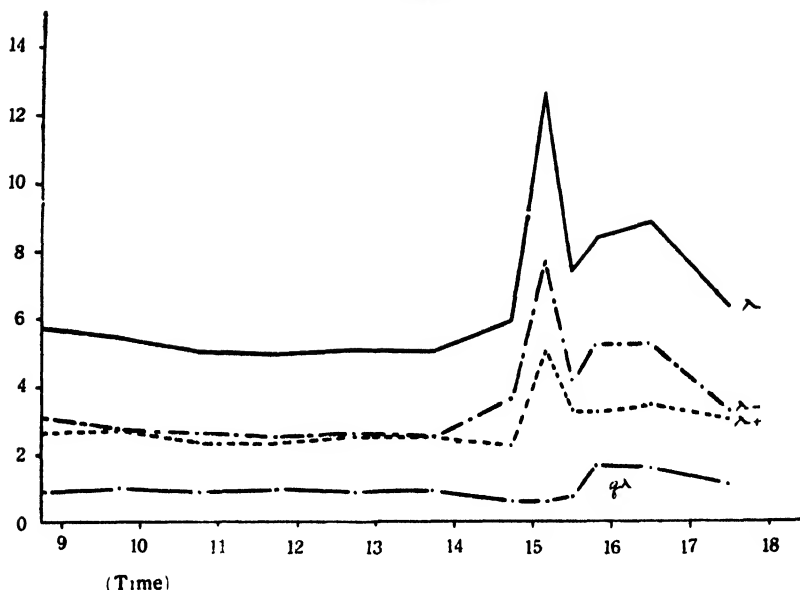


both polar conductivities during the thunderstorm of June 26. In the morning it was fine calm hot weather and the conductivity was about 2.6×10^{-4} e. s. u., very steady, rather lower value than the monthly normal and both $\lambda+$ and $\lambda-$ were nearly same, the q_λ was 1. At about $11\frac{1}{2}^h$ a very high breeze of N was appeared when cumulus cloud was occurred and both polar conductivity began to diminish. At $12\frac{1}{2}^h$ the whole sky was covered by cumulonimbus and the λ became 1.68×10^{-4} e. s. u. The thunderstorm began at $13^h 10^m$, then both polar conductivities tended to increase rapidly, the negative increased more than the positive and the q_λ became into 0.5 at $13^h 30^m$.

Soon after it was changed into very heavy rain, then firstly the $\lambda+$ suddenly jumped to 4.35×10^{-4} e. s. u. and after having made one oscillation the $\lambda-$ also jumped to extremely high value of 6.07×10^{-4} e. s. u. and as soon as the rain became moderate at $14^h 50^m$ both $\lambda+$ and $\lambda-$ were getting back to the ordinary value again. At $15^h 20^m$ the downfall was over.

Fig. 10_B No. 2 is also the graph of the thunder of August 14. In this case it was rather windy and the conductivity was rather high in the morning. At $13^h 45^m$ it became slight rain and the thunder

Fig. 10_B
No. 2. August 14.



was began. At first the $\lambda-$ was increased though the $\lambda+$ was considerably decreased, but when the rain became very heavy both polar conductivity jumped up to very high values and back again when it became slight rain.

It is noted from the above two thunder-storms that during heavy rain not only the $\lambda-$ but also the $\lambda+$ became a very high value and sometimes make oscillations and that such great change of the conductivity recovers back again as soon as the rain becomes light. Such changes of the conductivity during thunderstorm may be caused not only by the increase of ions produced by splashing of downfall known as Lenard effect and the decrease of the dust-nuclei as discussed by NOLAN,⁽¹⁸⁾ but also by the polarization effect due to the sudden great change of the atmospheric potentials which is often reversed during thunderstorm as indicated by WILSON⁽¹⁹⁾ and his successors.

TABLE X_A

Date			γ	$\gamma -$	$\gamma +$	q_{λ}	R	Time of beginning
1931	June	25	4.18	2.25	1.93	0.86	0.99	14.00
		26	2.64	1.32	1.32	1.00	0.62	13.10
	July	2	3.43	1.70	1.73	1.02	1.06	13.45
		9	3.23	1.70	1.53	0.90	1.00	14.45
		10	2.97	1.54	1.43	0.93	0.92	13.15
		11	1.71	0.82	0.89	1.10	0.53	13.30
		22	3.14	1.51	1.63	1.08	0.97	14.45
		23	3.72	1.82	1.90	1.04	1.15	15.00
		24	2.43	1.40	1.03	0.74	0.75	16.00
		25	2.83	1.71	1.12	0.66	0.88	13.45
		27	2.54	1.48	1.06	0.72	0.79	14.30
		28	2.28	1.12	1.16	1.04	0.71	15.30
	Aug.	1	5.14	3.00	2.14	0.71	1.35	13.15
		5	4.64	2.68	1.96	0.73	1.22	14.45
		14	5.43	2.83	2.60	0.92	1.43	13.45
		20	2.68	1.32	1.36	1.03	0.70	13.30
	Sept.	3	1.93	1.02	0.91	0.89	0.57	14.45
		7	2.47	1.21	1.26	1.04	0.72	14.30

TABLE X_A

Date			γ	$\gamma -$	$\gamma +$	q_{λ}	R	Time of beginning
1932	March	10	3.51	1.49	2.02	1.36	0.56	15.45
		11	3.31	1.01	2.30	2.28	0.53	15.30
		12	4.43	1.65	2.78	1.68	0.71	13.45
		14	9.38	4.26	5.12	1.30	1.51	16.45
		18	4.93	2.15	2.78	1.29	0.79	16.30
	April	5	7.03	3.34	3.69	1.11	1.85	13.00
		6	9.59	4.36	5.23	1.20	2.52	12.45

TABLE X_A

Date			λ	$\lambda -$	$\lambda +$	q_{λ}	R	Time of beginning
1932 April	13		3.30	1.70	1.60	0.94	0.87	14.30
	20		2.22	1.26	0.96	0.76	0.58	15.45
May	3		2.12	0.96	1.16	1.21	0.59	13.45
	9		2.79	1.54	1.25	0.81	0.77	13.45
	16		3.68	1.79	1.89	1.06	1.01	13.30
	19		3.38	1.70	1.68	0.99	0.93	14.30
	2		2.55	1.23	1.32	1.07	0.60	14.30
June	14		3.50	1.37	2.13	1.56	0.83	13.45
	15		3.52	1.40	2.12	1.52	0.83	13.30
	18		5.02	1.94	3.08	1.59	1.18	14.00
	5		4.52	2.26	2.26	1.00	1.40	14.45
July	6		2.42	1.16	1.26	1.09	0.75	14.45
	8		4.32	2.16	2.16	1.00	1.39	14.30
	9		4.60	2.30	2.30	1.00	1.43	14.55
	13		4.02	1.88	2.14	1.14	1.25	13.45
	18		4.68	2.34	2.34	1.00	1.45	15.45

TABLE Xa
Thunderstorm
No. 1. June 26, 1931

Time	λ	$\lambda -$	$\lambda +$	q_{λ}	Hum.	Wind vel. direction	Cloud.	Press.	Temp.	N. B.
8.45	2.50	1.30	1.20	0.94	83	0	—	755.0	28.4	
10.25	2.80	1.35	1.45	1.08	70	0	—	754.1	30.5	
11.30	1.75	0.83	0.92	1.11	71	0.3	N	754.1	31.0	
12.00	1.73	0.81	0.92	1.14	76	0.3	N	754.1	30.8	
12.30	1.68	0.79	0.89	1.13	82	0.3	N	754.1	30.1	
13.00	1.71	0.80	0.91	1.14	85	0.4	N	754.2	29.8	
13.30	3.93	2.38	1.55	0.51	87	0.5	N	754.2	29.5	Rain began
14.00	8.03	3.68	4.35	1.18	93	0.5	NE	754.2	28.5	Rain
14.10	6.08	3.04	3.04	1.00	91	1	E	754.3	27.5	Very heavy
14.20	8.50	6.07	2.43	0.40	91	1	ESE	754.3	27.5	Very heavy
14.30	10.65	5.79	4.86	0.84	88	1	E	754.3	27.0	Very heavy
14.50	4.51	2.04	2.47	1.21	90	0	—	754.3	27.5	Light rain
15.20	2.86	1.43	1.43	1.00	84	0	—	754.3	27.5	Rain end
15.45	2.97	1.48	1.49	1.01	86	0	—	754.3	27.7	
16.00	3.53	1.73	1.80	1.05	87	0.2	E	754.3	28.0	
16.30	3.53	1.67	1.92	1.15	87	0.2	E	754.2	27.8	
17.30	3.62	1.68	1.94	1.15	88	0.3	E	754.2	27.6	
18.00	3.32	1.60	1.72	1.08	87	0	—	754.2	27.4	

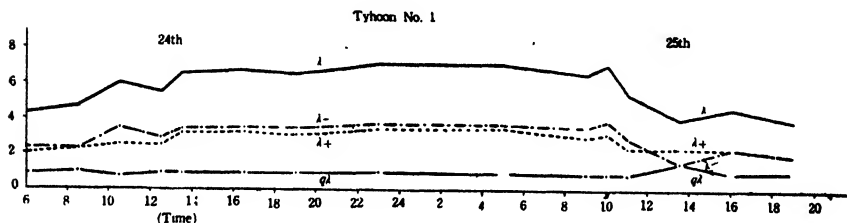
TABLE X_B
Thunderstorm
No. 2. August 14, 1931

Time	λ	$\lambda -$	$\lambda +$	q_{λ}	Hum.	Wind vel. direction	Cloud	Press.	Temp.	N. B.
8.45	5.75	3.09	2.66	0.86	66	3.2 E	4	752.6	30.3	
9.45	5.48	2.74	2.74	1.00	61	2.6 ESE	4	752.6	30.5	
10.45	5.05	2.66	2.39	0.90	63	3.5 ESE	4	752.6	30.5	
11.45	4.96	2.57	2.39	0.97	61	4.0 ES	4	752.5	30.7	
12.45	5.14	2.66	2.48	0.93	59	3.8 E	3	752.3	31.7	
13.45	5.05	2.57	2.48	0.96	70	3.1 E	4	746.9	30.8	Very slight rain
14.45	5.92	3.66	2.26	0.62	78	2.2 E	6	746.7	30.6	Slight rain
15.10	12.67	7.65	5.02	0.66	84	2.0 E	9	746.5	29.7	Very heavy
15.30	7.38	4.13	3.25	0.78	82	4.3 E	9	746.5	29.9	Moderate
15.50	8.43	5.20	3.23	1.60	88	3.1 E	8	749.6	29.0	Heavy
16.30	8.81	5.32	3.49	1.52	88	2.7 E	7	749.8	28.7	Heavy
17.30	6.32	3.23	3.04	1.07	88	2.2 E	7	750.9	28.0	Slight

11. Variation during Typhoon

During the course of observations we twice got the results of measuring the variations of the conductivity in typhoons, one of them was the typhoon on August 24th and 25th, which centre of the low passed northward slowly in the eastern ocean at about 95km distant away from Taihoku where the barometer began to fall gradually from the night of the 23rd, and became the minimum 744.2 mm at about 3^h of 25th. The wind was most reached strong from 10^{1/2}^h to 20^h on the 24th, and the total precipitation was 180.7 mm. The result of observation of the λ and the meteorological conditions are in the Table XI No. 1.

Fig. 11

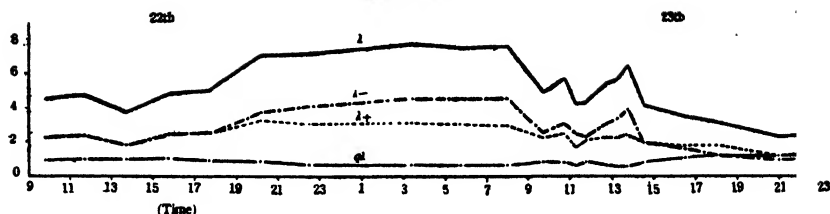


Before the storm the λ was about 3.2×10^{-4} e. s. u. and as the q_λ was nearly unity, both polar conductivities were equal each other, but when the barometer was falling down and the wind and rain were becoming intense the λ was getting to be increased; and during the storm the λ was above 6×10^{-4} e. s. u., the $\lambda-$ was far larger than the $\lambda+$, so that the q_λ was less than unity. At near the minimum of the pressure the λ took its maximum, though the conductivity was seemed to be fluctuated, much depending to the intensity of rain and wind. After the storm the λ was so decreased that it became about 2×10^{-4} e. s. u. and the negative conductivity was rendered much smaller than the positive, therefore the q_λ grew into greater than one.

Regarding another typhoon which passed in a NNW direction very near Taihoku on September 23rd. the variation of the conductivity and meteorological elements are given in the Table XI No. 2.

Fig. 11

Typhoon No. 2



As clearly seen in the Table the variation of the λ was similar with the typhoon of August, as that before the storm the λ was about 4×10^{-4} e. s. u., the q_λ was nearly one; then during the storm conductivity was greatly increased and the q_λ was much decreased, but after the storm the λ was recovered back and became much smaller than that of before the storm and the positive polar conductivity predominated. The maximum of the λ was near the minimum of the barometer.

These variations should be explained by the influence of intensity of rain and wind which caused the Lenard effect and which controlled the number of the dust nuclei and the respiration of soil.

TABLE XI
TyphoonNo. 1 (Total precipitation 180.7 mm¹)

Date	Time	λ	$\gamma -$	$\gamma +$	q_A	Hum.	Wind vel. direct.	Cloud.	Press.	Temp.	N. B.
23	12.00	3.26	1.74	1.52	0.87	72	1.3	ESE	752.2	30.8	
	14.00	3.01	1.62	1.39	0.86	73	1.2	S	75.22	31.1	
	20.00	3.39	1.65	1.74	1.05	86	1.4	W	751.6	27.1	
24	6.00	4.33	2.30	2.03	0.89	83	3.4	W	750.0	25.0	Slight rain
	8.30	4.66	2.33	2.33	1.00	86	5.9	W	747.1	25.0	Rain
	10.30	5.99	3.42	2.59	0.75	93	10.0	W	746.8	25.7	Heavy rain
25	12.30	5.44	2.87	2.57	0.90	93	10.0	W	746.9	25.8	Rain
	13.30	6.53	3.42	3.11	0.91	91	7.6	W	745.6	25.6	Heavy rain
	16.30	6.72	3.51	3.21	0.91	94	9.0	W	745.4	25.5	Heavy rain
25	19.00	6.56	3.47	3.03	0.89	94	9.8	W	754.1	25.2	Heavy rain
	23.00	7.03	3.64	3.45	0.95	96	4.6	W	744.9	24.8	Rain
	5.00	7.08	3.65	3.42	0.93	64	5.5	W	745.0	25.1	Heavy rain
25	9.00	6.42	3.50	2.92	0.83	96	5.5	W	745.0	25.1	Rain
	10.00	7.01	3.82	3.19	0.83	95	4.5	W	745.2	25.2	Rain
	11.00	5.29	2.92	2.37	0.82	94	4.0	W	745.3	25.6	Slight rain
25	13.30	3.96	1.57	2.39	1.52	93	2.8	W	745.5	25.7	Slight rain
	16.00	4.58	2.32	2.26	0.97	92	3.3	W	749.0	25.4	Slight rain
	19.00	3.84	1.92	1.92	1.00	88	1.8	W	750.4	25.0	Slight rain

No. 2. (Total precipitation 91.8 mm)

Date	Time	γ	$\gamma -$	$\gamma +$	q_A	Hum.	Wind vel. direct.	Cloud.	Press.	Temp.	N. B.
22	9.45	4.42	2.22	2.20	0.99	60	2.7	E	755.6	29.1	
	11.45	4.78	2.39	2.39	1.00	83	2.7	ESE	755.4	26.5	Rain
	13.45	3.68	1.84	1.84	1.00	78	2.3	E	754.0	27.9	Slight rain
	15.45	4.87	2.40	2.47	1.03	83	1.9	ESE	754.1	26.9	Rain
	17.45	4.98	2.51	2.47	0.99	86	5.8	ESE	750.6	25.8	Rain
	20.10	7.06	3.72	3.34	0.90	88	9.7	ESE	747.2	25.7	Heavy rain
	22.30	7.12	4.03	3.03	0.74	88	9.7	ESE	746.1	25.7	Heavy rain
	3.20	7.64	4.51	3.13	0.69	86	6.2	E	744.7	25.6	Rain
	5.45	7.48	4.56	3.12	0.68	88	9.3	E	745.0	25.3	Heavy rain
	8.00	7.58	4.58	3.00	0.65	87	8.0	E	745.2	25.2	Heavy rain
23	9.45	4.83	2.57	2.26	0.88	89	6.4	E	745.4	24.8	Rain
	10.45	5.70	3.13	2.57	0.82	92	4.4	E	745.3	25.4	Rain
	11.15	4.22	2.57	1.65	0.64	93	5.0	WSW	745.0	24.8	Rain
	11.45	4.33	2.30	2.03	0.89	94	2.5	SW	745.8	25.0	Rain
	12.45	5.43	3.13	2.30	0.73	92	3.3	NNE	746.3	24.2	Rain
	13.15	5.68	3.42	2.26	0.66	90	2.5	E	746.2	24.2	Rain
	13.45	6.55	3.98	2.57	0.65	93	1.1	ENE	748.4	24.1	Heavy rain
	14.30	4.16	2.16	2.00	0.93	90	1.0	N	749.6	24.1	Slight rain
	16.30	3.53	1.68	1.84	1.09	89	0.6	NW	749.7	24.0	Rain end
	18.00	3.20	1.36	1.84	1.35	88	0.4	N	752.8	24.0	
21.00	21.00	2.46	1.10	1.36	1.27	89	0.6	N	753.6	24.1	
	23.00	2.65	1.10	1.55	1.41	87	0.7	N	754.4	24.0	

XII. Conclusion

The results presented in this paper lead to the following conclusion :—

Conductivity is more fluctuable in summer than in winter and the negative polar conductivity is more unsteady than the positive one which is more stable in winter than in summer.

When the winter type and the summer type are alternated the atmospheric electrical conditions are very unsteady and the variance is largest, and the value of the conductivity takes a great change before and after this time.

The deviation in the diurnal oscillation of the conductivity is greater in winter than in summer, even though its variance is far smaller in winter than in summer.

The associations between conductivity and meteorological elements, except wind velocity, are not so simple and apparently seem to differ by season. Such different relationship in each season may be caused from that, as the correlated relationship between the elements are different by season and much complicated each other, so that the influence of the single meteorological element on conductivity is not clear, but only the resultant effect of the element with others is shown and the principal meteorological element which characterize the season by affecting great influence upon other elements is not same for each season. The influences of such temperature and pressure to conductivity shows different fashion for winter type and summer type, but the effect of wind velocity is to increase the conductivity for both types. The difference of the conductivity due to the wind directions may be the character of the locality which depends on the difference of ionic state and purity of air in each directions of the winds.

By the method of partial correlation for finding the effects of a single elements it can be known that the principal influence is wind velocity in any season, but only in morning of summer the largest influence is temperature.

The effect of rain depend largely on its intensity, and heavy rain causes often abnormal increases of conductivity, though drizzle rain lets it decrease. The wetness of soil after rain affects to the content of emanation in air, therefore results to reduce conductivity. The conductivity during thunderstorm and typhoon undergoes abnormal variation not only for the $\lambda -$ but also for the $\lambda +$, which may be caused by the polarization-effect of the atmospheric electric field and by the influence of wind and rain.

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Notation in the tables and figures

$\lambda, \lambda -, \lambda +$ = Total polar conductivity in unit of 10^{-4} e. s. u.

$$q_{\lambda} = \lambda + / \lambda -$$

n = Number of individuals used.

P = Pressure in mm.

T = Temperature in C.

F = Relative humidity (Hum.).

W. v. = Wind velocity (m/sec).

W. Direct. = Wind direction.

B = Cloudiness.

γ = Correlation coefficient in unit of $1/100$

Pe = Probable errors of γ in unit of $1/100$

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